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EFFECT OF SEVERAL CALCIUM SALTS ON THE UTILIZATION OF LACTOSE [1]

HELEN S. MITCHELL, GLADYS M. COOK AND KATHERINE L. O'BRIEN
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TWO FIGURES

(Received for publication May 27, 1939)

The relationship of lactose and calcium metabolism has long been recognized, especially the role of lactose in promoting better absorption of calcium from the intestinal tract. The utilization or retention of calcium has been studied after oral administration of different forms of calcium and as influenced by different forms of carbohydrate in the ration, but little attention has been given to the possible influence of the various calcium salts on the utilization of other dietary constituents. The present observations in this field were incident to experiments being conducted in this laboratory on the cataractogenic action of lactose as influenced by inorganic constituents of the ration. The calcium salts used were those most commonly employed in present day calcium therapy. Observations in this laboratory indicate considerable variation in the effect of different calcium salts on the digestion or absorption of lactose in rats.

EXPERIMENTAL

All animals were started on experimental rations at between 25 and 30 days of age and were so distributed as to afford adequate litter-mate controls. The basal rations used were complete in the usual sense, i.e., in quantity and proportion of

[1] Published as contribution no. 347 of the Massachusetts Agricultural Experiment Station.

ingredients and in vitamin supplements. They varied only in the type of carbohydrate incorporated in the ration and in the calcium salts used as supplements. All rations were fed ad libitum with accurate records of food intake. The composition and designation of the specific rations were as follows:

TABLE 1

Experimental rations

Composition	*60% lactose*	*65% starch*	*65% other CHO*	*25% galactose*
Lactose	60			
Starch (corn)	5	65		40
Dextrin				
Sucrose			65	
Glucose				
Galactose				25
Casein (technical)	15	15	15	15
Crisco	9	9	9	9
Cod liver oil	2	2	2	2
Salt mixture (O & M)	4	4	4	4
Yeast (dry brewers)	5	5	5	5

Calcium salts were added to these rations in quantities calculated to supply respectively 1.0 or 0.5% of calcium in addition to the 0.5% already present as part of the incorporated salt mixture. The calcium salts used were the carbonate, tricalcium phosphate, citrate, lactate, levulinate and gluconate, and were of C.P. or U.S.P. grade. Since lactose was the carbohydrate of primary interest in this investigation, by far the most data were accumulated with rats on the 60% lactose ration with and without the added salts. Starch, dextrin, sucrose, glucose and galactose rations plus added calcium salts served as controls.

In the first series of experiments, 1.0% of calcium was added to the basal ration as follows: 2.6 gm. tricalcium phosphate, 2.5 gm. calcium carbonate, 8.0 gm. (later reduced to 6.0 gm.) calcium lactate, 11.2 gm. calcium gluconate respectively per 100 gm. of ration. Careful record was kept of survival, growth, food intake, evidence of diarrhea, incidence and rate of cataract development and blood sugar level during absorption. All experiments ran for 9 weeks or longer.

The results of feeding the various calcium salts incorporated in a lactose ration are shown in the table (table 2). The growth, survival and cataract development of rats receiving the carbonate or phosphate approximated those on the basal lactose ration, and the diarrhea was somewhat less. Blood sugar tended to be slightly higher in rats receiving the carbonate or phosphate (150 mg. and 146 mg. per 100 cc.), respectively as compared with litter-mate controls on the 60% lactose ration (135 mg. per 100 cc.). The rats receiving the calcium lactate or gluconate showed definite toxic symptoms, retarded growth, poor survival and moderate to severe diarrhea in the case of the gluconate. More striking still was the lower blood sugar (103 mg. per 100 cc.) and absence of lenticular changes in rats on the gluconate in contrast to high blood sugar and usual incidence of cataract in all other groups on lactose rations. Collectively, these findings would indicate that the gluconate, and to a lesser extent the lactate, interfere in some manner with the utilization of lactose.

Calcium salts incorporated in starch, dextrin, sucrose, galactose and glucose rations at the 1.0% level were well tolerated by rats with no untoward effects. The good growth and general well-being of rats on these five carbohydrates were evidence that calcium salts as such, at the levels fed, were not seriously toxic or detrimental, nor were there any significant differences in the effect of individual salts (fig. 1). Growth efficiency calculated from the total weight gained in 9 weeks divided by the total food eaten in the same period was remarkably constant for the different sugars, irrespective of the calcium salts added. Since expense necessitated the termination of galactose experiments when cataract developed, growth efficiency in this group was based upon data for a shorter period but showed no variation with added calcium salts. The rapid and constant time for development of cataract on 25% galactose rations with or without added calcium salts is further evidence that absorption of this single sugar was not inhibited. Previous work by Mitchell et al.

TABLE 2

Effect of calcium salts on the utilization of lactose

RATION	NUMBER OF RATS	SURVIVAL PER CENT	GAIN IN WEIGHT IN 9 WEEKS		SEVERITY OF DIARRHEA EARLY — LATE	INCIDENCE OF CATARACT		BLOOD SUGAR	
			Male	Female		Early changes	Mature cataract	Milligrams per 100 cc.	Number of determinations
			gm.	*gm.*		%	%		
1.0% calcium in added salts									
60% Lactose	32	94	111	92	+++—+	100	37	135	(82)
60% L — 2.6% $Ca_3(PO_4)_2$	33	96	96	85	++—0	100	42	150	(80)
60% L — 2.5% $CaCO_3$	14	100	73	75	+—0	100	39	146	(53)
60% L — 8.0% Ca lactate	4	0	..	..	+++	..	..	..	..
60% L — 6.0% Ca lactate	4	50	9	10	++—0	100	0		
60% L — 11.2% Ca gluconate	13	55	23	6	++++—++	0	0	103	(16)
70% Starch	15	100	145	111	0 0	0	0	113	(37)
0.5% calcium in added calcium salts									
60% Lactose	15	100	124	105	++—0	100	53	145	(29)
60% L — 1.3% $Ca_3(PO_4)_2$	6	100	112	67	+—0	100	8[1]	146	(10)
60% L — 2.4% Ca citrate	6	100	125	81	++—0	100	20[1]	156	(9)
60% L — 4.0% Ca lactate	9	100	81	79	+—0	100	45	151	(13)
60% L — 3.4% Ca levulinate	4	100	100	84	+—0	100	25[1]	127	(11)
60% L — 5.6% Ca gluconate	18	67	52	38	+++—++	22	0	100	(21)
60% L — 5.6% Na gluconate	8	38	104	113	++++—+++	0	0	106	(6)
65% Starch	11	100	202	122	0 0	0	0	113	(14)
65% S — 5.6% Ca gluconate	7	100	182	128	0 0	0	0	117	(11)
65% S — 5.6% Na gluconate	2	100	190	173	0 0	0	0	131	(6)

[1] Rats from cataract-resistant litters are included in these averages.

('37) indicates that lens injury correlates with high blood sugar (fig. 2).

In the second series of experiments, 0.5% of added calcium was given as 1.3 gm. tricalcium phosphate, 2.4 gm. citrate, 4.0 gm. lactate, 3.4 gm. levulinate and 5.6 gm. gluconate, per 100 gm. of food. The same type of observations was made as in the first series, plus a few bone ash determinations. (The percentage of ash in the dry fat-free bone was practically constant (68.4 to 70.2) for all rats, irrespective of the

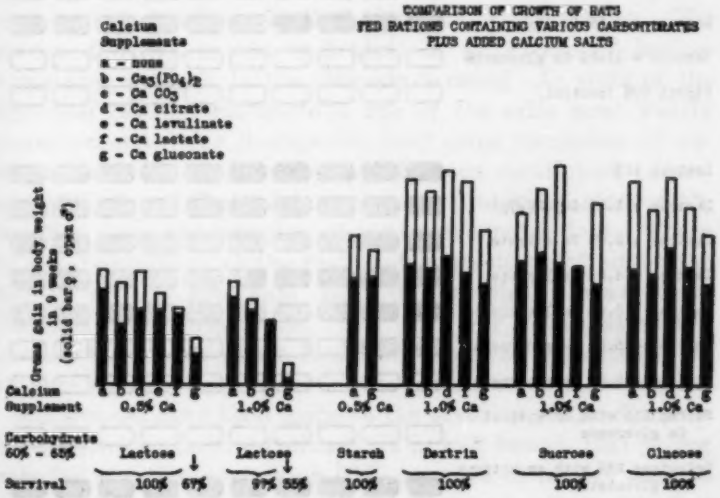

Figure 1

ration, and served to indicate that the calcium metabolism per se was not disturbed.) The results of the two series of experiments are similar, the second serving to verify the first but obviously modified because the salts were added at half the previous level to reduce mortality and allow more prolonged study of the metabolic abnormalities (table 2).

Again certain calcium salts, namely the phosphate, citrate and levulinate exerted no appreciable effect as evidenced by rate of growth, growth efficiency, survival, blood sugar level

or cataract development. The lactate at the lower 4.0 gm. level was not so toxic as in the first series, although growth was slightly retarded. Growth efficiency is somewhat lower on all lactose rations than on other carbohydrate but is not altered by the above salts except slightly in the case of the lactate.

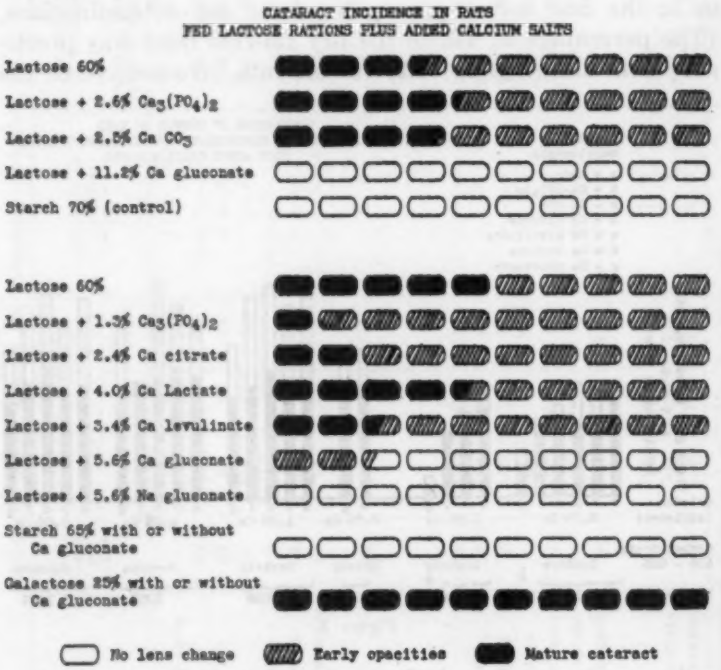
CATARACT INCIDENCE IN RATS
FED LACTOSE RATIONS PLUS ADDED CALCIUM SALTS
Lactose 60%
Lactose + 2.6% $Ca_3(PO_4)_2$
Lactose + 2.5% $CaCO_3$
Lactose + 11.2% Ca gluconate
Starch 70% (control)
Lactose 60%
Lactose + 1.3% $Ca_3(PO_4)_2$
Lactose + 2.4% Ca citrate
Lactose + 4.0% Ca Lactate
Lactose + 3.4% Ca levulinate
Lactose + 5.6% Ca gluconate
Lactose + 5.6% Na gluconate
Starch 65% with or without Ca gluconate
Galactose 25% with or without Ca gluconate
No lens change
Early opacities
Mature cataract

Figure 2

The calcium gluconate, however, still exerted its inhibitory effect when fed at the lower level (0.5% of calcium), and results were similar for three different brands.[2] Retarded growth and lowered growth efficiency, poor survival, severe

[2] The calcium gluconate was obtained from three different commercial sources: 1. Sandoz Chemical Works, Inc.; 2. Chas. Pfizer and Co., Inc.; 3. Pfanstiehl Chemical Company.

diarrhea, reduced blood sugar and slight if any lenticular changes indicated that the lactose was not being assimilated normally (fig. 2). The fact that both glucose and galactose fed as such resulted in as good growth efficiency in presence of the gluconate as without it indicates no interference with absorption of the simple sugars. The enzymatic hydrolysis of starch, dextrin and sucrose also appear to proceed normally. The failure of the blood sugar to show the usual rise during absorption when calcium gluconate was associated with lactose seemed to indicate that the calcium gluconate inhibited the enzymatic hydrolysis of the lactose. The severe diarrhea is also indicative of an excess of lactose remaining, as such, in the alimentary canal. In spite of the fact that calcium gluconate is one of the salts most widely recommenended for therapeutic use,[3] some indication of unfavorable reactions have previously been mentioned. Johnson ('35) found that calcium gluconate inhibited glucose absorption from the intestine of his experimental dogs. There is also recognition of the fact that large doses of either the lactate or the gluconate used therapeutically may cause diarrhea (correspondence, '38). In our own experience urinary calculi have been found in several of the rats fed on calcium gluconate and never in rats fed the other calcium salts.

References have been made to the severity of the diarrhea on the various lactose rations. It is well known that young rats fed a ration containing 50% or more of lactose tend to develop diarrhea, more severe in some than in others, but that they usually develop a tolerance or outgrow this tendency after a few weeks. An abrupt shift from a stock ration to a high level of lactose is frequently fatal to young rats. For this reason, a graduated proportion of a starch ration was mixed with the lactose ration for a few days at the beginning of these experiments in order to keep the rats alive until they developed some degree of tolerance. It has been notable throughout this experiment that most of the rats fed either of

[3] New and Nonofficial Remedies, 1938, p. 158. Useful Drugs, 1936, p. 69. Epitome of U. S. Pharmocopeia and National Formulary, 1937, p. 48.

the gluconate salts in a lactose ration continued to show almost as severe diarrhea after several weeks as at first; a few on calcium gluconate developed a degree of tolerance.

Since no other calcium salts investigated approached the gluconate in toxic or inhibitory action, the gluconate radical was brought under suspicion. Consequently, sodium gluconate was fed at a comparable level incorporated in a 60% lactose ration. The untoward effects were strikingly similar but rather more severe than with the calcium gluconate fed to litter-mate controls, i.e., the survival was poorer, diarrhea worse and lens changes absent, but in the few rats which did survive the rate of growth and growth efficiency improved after a few weeks in spite of a persistent diarrhea, a finding which cannot be readily explained. Thus it would appear that the gluconate radical, rather than the calcium, must be the part which intereferes with lactose activity in the alimentary canal. One may postulate some such phenomenon as 'competitive inhibition,' in enzyme action, due to similarity in structure of the lactose and the gluconate radicals. In vitro work on this phase of the subject is in progress but is not yet conclusive.

SUMMARY

1. Rats were fed an adequate ration containing 60% lactose, plus 1.0 or 0.5% of calcium added in the form of six different calcium salts, respectively, tricalcium phosphate, carbonate, citrate, lactate, levulinate and gluconate.

2. With the phosphate, carbonate, citrate and levulinate rats showed as good growth, less diarrhea in general, the same degree of galactemia and a similar incidence of cataract as on the plain 60% lactose ration. Calcium lactate was somewhat toxic at the higher level but at the lower level results were more nearly comparable to those with the other calcium salts mentioned above.

3. Calcium gluconate fed at the 1.0% calcium level resulted in poor survival, loss of weight, severe diarrhea and low normal blood sugar. At the 0.5% level of calcium survival

was better, but growth was poor, diarrhea moderate to severe, blood sugar a low normal and lenticular changes few if any.

4. Calcium gluconate exerted no apparent inhibitory effect upon the absorption or utilization of starch, dextrin, sucrose, glucose or galactose.

5. Sodium gluconate exerted a deleterious effect similar to but more severe than calcium gluconate when fed in a lactose ration.

6. These criteria indicate that the gluconate radical may interefere with lactase activity in the intestinal tract. The phenomenon of 'competitive inhibition' in enzyme action is postulated as a possible explanation of these findings.

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FATTY LIVERS AS A RESULT OF THIAMIN ADMINISTRATION IN VITAMIN B_1 DEFICIENCY OF THE RAT AND THE CHICK [1, 2]

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FOUR FIGURES

(Received for publication May 13, 1939)

INTRODUCTION

While investigating the pathology of an uncomplicated vitamin B_1 deficiency in the rat it was discovered ('38) that if the animals were restored to normal by the addition of thiamin to the vitamin B_1 deficient ration a hydropic degeneration appeared in the liver cells. Rats suffering from vitamin B_1 deficiency, if restored to normal by feeding them a ration rich in fat and free of carbohydrate, had retained a normal liver cell structure. These observations led to more thorough histologic and chemical studies concerning the effect of thiamin therapy in B_1 avitaminosis. Experiments were carried out with growing rats and with growing chicks.

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

[2] These studies were aided in part by a grant from the Wisconsin Alumni Research Foundation and in part by the Works Progress Administration.

EXPERIMENTAL

Part I. Studies on the rat

Ration 112 of Arnold and Elvehjem ('38 a) was used to produce vitamin B_1 deficiency. Its composition was as follows:

Sucrose	62
Purified casein	18
Autoclaved peanuts	10
Autoclaved A.B. yeast	4
Salts I	4
Liver extract Factor W concentrate	2 liver extract
Percomorph oil	3 drops weekly

An experiment was first made to determine the protective ability of various agents against the fatty livers which result when rats are relieved of vitamin B_1 deficient symptoms by thiamin supplements. Litter mate weanling albino rats were depleted of their vitamin B_1 stores. When severe deficiency symptoms were apparent, two animals which served as controls were removed for study. The remaining animals were removed after having received various supplements over a 1-week period. The dietary supplements and the results of this experiment are summarized in table 1.

The livers were examined microscopically with the use of H and E stain as well as with osmic acid (Romeis, '32) for the detection of fat. Occasionally pancreas, kidney and adrenal gland tissue was also examined.

After histologic specimens had been obtained the remainder of the livers was weighed, dried at 100°C. and then re-weighed. The difference in weight represented moisture. Lipids were determined by grinding the dried samples and extracting for 18 hours with ethyl ether.

Supplements of thiamin resulted in an increase in liver lipids as is shown in table 1. An increase in liver size and weight also resulted from thiamin therapy. The increase in liver weight could not be completely accounted for from the rise in lipids. Histologic observations substantiated the chemical data since all cases which had received thiamin

showed hydropic degeneration and fat metamorphosis in the liver. At autopsy these livers were observed to be enlarged and light in color. The control animals (vitamin B_1 deficient) had a normal liver cell structure. It is obvious that none of the supplements tested were protective against the fatty liver condition.

Since an inanition accompanies severe vitamin B_1 deficiency, it seemed advisable to test the effect of thiamin on liver fat in starvation. To this end four litter mate weanling rats were

TABLE 1

The effect of thiamin administration upon liver structure and composition in the B_1 deficient rat

LOT	NUMBER OF ANIMALS	DIETARY SUPPLEMENTS	LIVER PER CENT OF BODY WEIGHT	LIPIDS	MOISTURE	DEGREE OF HISTOLOGIC DAMAGE
				%	%	
I	2	None	3.00	6.46	73.2	0
			3.05	7.28	70.4	0
II	2	100 μg. thiamin/100 gm. of ration	5.25	9.13	73.3	+++
			6.00	9.30	70.6	+++
III	2	100 μg. thiamin/100 gm. + lipocaic = 10% fresh pancreas	6.10	10.35	75.6	++
			5.50	9.28	70.1	+++
IV	2	100 μg. thiamin/100 gm. + 5 mg. choline/rat/day	5.72	8.33	71.1	+++
			5.53	8.38	69.0	+++
V	2	Isocaloric replacement of carbohydrate with fat + 20 μg. thiamin/100 gm.	5.88	12.22	68.3	++
			4.99	12.30	70.6	++

starved. Two were injected intraperitoneally with 100 μg. of thiamin on the third day of starvation. All animals were removed on the fourth day. Microscopic examination of the livers revealed only a slight fat reaction in the cells near the central veins in all these cases. These observations suggest that the inanition which usually accompanies severe B_1 avitaminosis is not concerned with the fatty livers resulting from thiamin therapy.

It has been suggested by Sure ('35) that an antagonistic relationship exists between thiamin and thyroxin. The effect

of the latter on the fatty livers obtained with thiamin therapy in B_1 avitaminosis was next studied. Four young rats received 5 mg. of desiccated thyroid daily after having been kept on ration 112 for 4 weeks. This supplement produced severe B_1 deficiency symptoms before the end of the fifth week. Two of these animals were then removed while the remaining two were removed 24 hours after each had received 400 μg. of thiamin intraperitoneally.

H and E stained sections, as well as osmicated sections revealed a normal liver cell structure in all cases. These results indicate that the increased rate of metabolism induced by desiccated thyroid feeding was responsible for the prevention of the fatty liver condition occurring in thiamin therapy.

Part II. Studies on the chick

To determine if this hitherto unnoticed liver pathology could be produced in other species, studies were next made on the chick. Vitamin B_1 deficiency was produced by feeding 1-day-old chicks ration 242A of Arnold and Elvehjem ('38 b). This ration consists of:

A. Autoclaved portion		*B. Untreated portion*	
Ground yellow corn	57	Dried whole liver	1
Wheat middlings	25	Iodized salt	1
Crude acid precipitated casein	12	$CaCO_3$	1
		$Ca_3(PO_4)_2$	1
		Cod liver oil	2

In a preliminary experiment with six chicks it was shown that thiamin, when given to vitamin B_1 deficient chicks over a period of 7 days in daily oral doses of 50 μg., produced enlarged light colored livers. Microscopic examination showed that fatty metamorphosis was present.

In the next experiment an attempt was again made to determine the protective value of various agents. The experimental procedure was as follows:

Lot	*Number of animals*	*Dietary regimen*
I	4	Basal ration + 0.05% desiccated thyroid
II	4	Basal + 5 mg. choline/chick/day
III	4	Basal + 30% acid precipitated casein

Two of the birds from each lot served as controls and were removed when severely vitamin B_1 deficient. The remaining birds were given intraperitoneal injections of 400 μg. of thiamin when in the severely deficient state and were sacrificed 48 hours later.

Previous results obtained with the rat were substantiated since no liver pathology resulted in the birds receiving both desiccated thyroid and thiamin. Neither choline nor a high level of casein in the diet protected the livers of these birds against fatty metamorphosis. All the controls (no thiamin) had normal livers.

TABLE 2

The effect of thiamin (or co-carboxylase) on liver glycogen in polyneuritic chicks

CHICK	DIETARY SUPPLEMENTS	DAYS ON THIAMIN THERAPY	PER CENT GLYCOGEN	DEGREE OF HISTOLOGIC DAMAGE
1225	20 μg. thiamin + 5 mg. choline	2	2.23	+
1227	200 μg. thiamin + 5 mg. choline	2	9.81	++
1228	200 μg. thiamin + 5 mg. choline	2	2.63	+++
1230	200 μg. thiamin + 5 mg. choline	2	8.86	+++
1231	400 μg. thiamin + 5 mg. glucose	1	3.14	+++
1235	500 μg. co-carboxylase	4	7.82	+++
1317	200 μg. thiamin + 0.08 units insulin	1	10.13	+++
1319	200 μg. thiamin + 0.08 units insulin	1	9.97	+++
(7 chicks)	(B_1 deficient, no supplement)	..	Av. 0.90	0

Further chemical studies were made on the fatty livers resulting from thiamin therapy in polyneuritic chicks in an attempt further to elucidate the nature of the structural changes. Table 2 presents some typical results of experiments in which it was attempted to correlate liver glycogen content with histologic observations. In these studies the dietary supplements were injected intraperitoneally. The birds were killed by decapitation and portions of liver immediately homogenized in alcoholic KOH solution for glycogen determination (modified Kerr method, '36).

It is apparent that a marked increase in liver glycogen occurs with thiamin therapy in polyneuritic birds. It is also obvious that insulin seemed to increase the liver glycogen de-

position when given along with thiamin and that it did not protect against fatty metamorphosis. Co-carboxylase was as effective as thiamin in producing the typical fatty livers.

The severity of the pathologic changes could not be correlated with the glycogen storage since livers with 3 to 4% glycogen were as severely affected as those with 8 to 10% glycogen.

In subsequent studies more complete chemical analyses were made. Some typical results are presented in table 3. Since in previous experiments the birds had been continued on the basal diet after thiamin therapy, it was also of interest

TABLE 3

The effect of thiamin on average liver composition

NUMBER OF BIRDS	RATION	LIVER PER CENT OF BODY WEIGHT	MOISTURE	EtOH EXTRACT	MG. P IN EtOH EXTRACT PER GRAM DRY LIVER (PHOSPHOLIPID P)	PROTEIN
			%	%		%
12	242A + 300 μg. thiamin	3.91	71.70	8.67	3.43	12.20
8	242A only	2.50	73.20	7.44	5.03	17.80
5	242A + 300 μg. thiamin [1]	2.17	73.70	8.32	4.48	17.50

[1] Autopsied in starvation 24 hours after the administration of thiamin.

to see if the typical enlarged fatty livers would result if the polyneuritic birds were starved after thiamin therapy. Typical polyneuritis was produced as usual. The thiamin was injected intraperitoneally 24 hours before autopsy.

At autopsy liver samples were preserved for histologic study. The remaining liver tissue was dried at 100°C. for moisture determination. The dried samples were then extracted six times with hot ethyl alcohol. Phosphorus was determined on aliquots of this extract and is represented as phospholipid phosphorus. The protein was calculated from the nitrogen in the residue after alcohol extraction.

When calculated on a percentage basis, the fat content in the livers of thiamin treated birds was only slightly higher than that of the controls. However, since the livers of birds

receiving thiamin plus basal ration were hypertrophied, it is obvious that the total fat in the livers of these birds was markedly increased over that of the controls. Similarly the total moisture was greatly increased with thiamin therapy. On the other hand, the total protein and phospholipid values compare favorably with the controls although they are lowered in

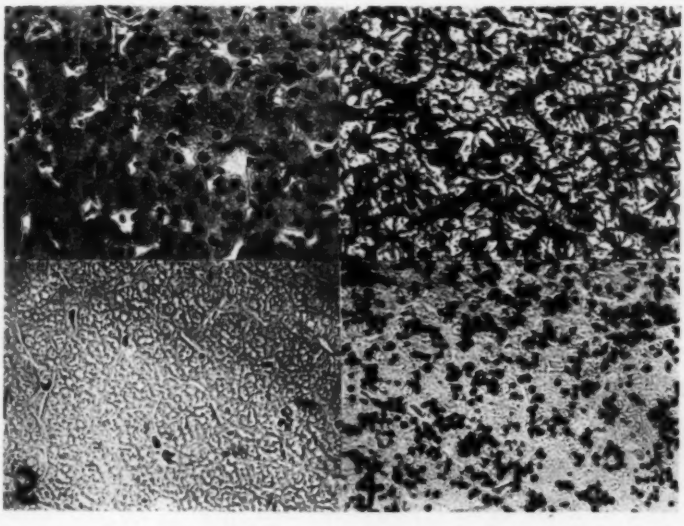

Fig. 1 Chick no. 1407. Vitamin B_1 deficient. Normal liver cell structure. H. and E. stain. × 440.

Fig. 2 Chick no. 1407. (Same as figure 1.) No fat staining. Maximov's osmic acid stain. × 440.

Fig. 3 Chick 1404. Vitamin B_1 deficient chick injected with 300 μg. of thiamin 24 hours before autopsy. Hydropic degeneration. H. and E. stain. × 440.

Fig. 4 Chick 1404. (Same as figure 3.) Fatty metamorphosis. Maximov's osmic acid stain. × 440.

the thiamin treated birds when calculated on a percentage basis. Similar phospholipid changes were observed by Flock ('39) who studied fatty livers in geese. From these data it would seem that the abnormal liver composition induced by thiamin therapy is largely due to storage of exogenous food

substances since the group which had received thiamin and then had been starved for 24 hours had a liver composition comparable to the controls. However, in this group there was also a slight increase in the per cent of liver fat.

Histologic studies on these livers revealed that the birds receiving the basal ration only had normal livers (figs. 1, 2). Typical hydropic and fatty liver changes were present in the two groups receiving thiamin (figs. 3, 4). However, the changes were much less severe in the group which had not had access to food after thiamin therapy.

DISCUSSION

The histologic changes which appeared in the livers as the results of thiamin therapy in vitamin B_1 deficient animals are of interest since this vitamin is rather extensively used clinically. Cowgill ('38) and others have used thiamin at rather high levels and failed to observe any adverse effects. Cowgill states that no toxic symptoms were observed in doses 25,000 or more times the estimated daily human requirement. Recently Steinberg ('38) has observed Herpes Zoster resulting in patients treated with large doses of thiamin. He suggested that thiamin in large doses irritates peripheral nerve plates. With few exceptions, reported data would suggest a wide range of tolerance of this vitamin by the animal body. Doses of the vitamin used in the studies reported here are considerably below toxic levels as suggested by Molitar and Sampson ('36) and by Hecht and Wiese ('37). It is true that no gross manifestations of toxicity were noticed in the present study. An increased liver size and occasionally a slight atrophy of the pancreas were the only abnormalities noted at autopsy.

The results obtained in these studies are in agreement with Whipple and Church ('36) and McHenry ('37) who have also demonstrated that thiamin increases liver fat. The protective value of choline against liver fat accumulation as reported by McHenry could not be confirmed when choline was fed at a level of 5 mg. daily.

Whether the extreme histologic reactions in the liver resulting from thiamin therapy in B_1 avitaminosis would lead to permanent liver damage is still a question. Recent evidence with the chick indicates that there is a gradual resorption of the liver fat over a period of 6 weeks, even though thiamin is administered continuously. The fact that the animals made remarkable weight gains and showed no gross ill effects from the thiamin therapy would indicate that even though the liver tissue appears abnormal histologically, its function has not been seriously and permanently disturbed.

SUMMARY AND CONCLUSIONS

When thiamin was administered to vitamin B_1 deficient chicks or rats hydropic degeneration and fatty metamorphosis occurred in the parenchyma of the liver cells. This histologic reaction in the liver was not prevented by choline, lipocaic or by diets high in fat or in casein. Desiccated thyroid was effective in preventing the histologic reactions in the livers.

Chemical analyses on these livers showed that there was an increase in total fat, glycogen and moisture. The phospholipid and protein remained unchanged.

Thiamin therapy in experimental vitamin B_1 deficiency causes an excessive production of free fat in the liver cell which disrupts the normal cell structure.

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FACTORS INFLUENCING STORAGE OF PROTEIN WITH LOW-CALORIE DIETS

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TWO FIGURES

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The purpose of this report is to demonstrate a) the effects of different forms of low-calorie diets upon the metabolism of protein, and b) the protein-sparing action of between-meal feedings of carbohydrates with low-calorie diets of the same composition with respect to carbohydrate and fat, but different amounts of animal and vegetable proteins. The importance of knowledge of the different factors which may influence the metabolism of protein with low-calorie diets is obvious from the importance of protein in nutrition and from the many people who, for different reasons in health and in disease, must reduce their intake of food. Low wage incomes and obesity are examples in health; diabetes is an example of disease.

Haggard and Greenberg ('35) have shown that between-meal feedings increase the net muscular efficiency of the human body and that the increase is associated with an increase of carbohydrate metabolism. The observations in general fitted in with the well-known fact that mechanical work is best done with carbohydrates; it is done less efficiently with fats and least efficiently with protein. This, however, is not the only beneficial effect of between-meal feedings. The latter prevent the stomach from becoming empty and an empty

stomach may impair efficiency, regardless of an adequate caloric intake by production of fatigue, etc.—Carlson's accessory phenomena of hunger.

In general, urinary excretion of nitrogen is governed by the intensity of the amino acid metabolism and, in man, the metabolism of tissue protein is never in abeyance (Borsook and Keighley, '35). Even with nitrogen equilibrium, endogenous (tissue) protein was found to account for more than half of the total urinary nitrogen (Beard, '35). In the construction of low-calorie diets, consideration must, therefore, be given to the conditions which tend to conserve nitrogen and one of these is the intake of protein, quantitatively and qualitatively; retention of protein is more readily accomplished with liberal than with low-protein diets and, with the same intake of protein, more readily accomplished with animal than with vegetable proteins. It is also a well-known fact that the total amount of carbohydrate in the diet also influences the degree of protein storage (Lusk, '28; Sherman, '37). Recently, however, Larson and Chaikoff ('37) demonstrated another variable in the protein-sparing action of carbohydrates, namely, time. These authors have shown that carbohydrates exert their maximum protein-sparing action only when they are available at the time that the intensity of the protein metabolism is at its maximum and that the interval during which extra carbohydrate is able to exert any nitrogen-sparing action is limited to about 4 hours before and 4 hours after the ingestion of the regular meal. The findings, in general, fit in with the more recent observation of Cuthbertson and Munro ('39) that, regardless of the amount of carbohydrate and protein fed, whether the nitrogen balance was positive or negative depended upon the number of meals per day.

The data to be reported here are based upon experiences with eight different diets of diabetics during the last 20 years. As will be shown, they afford additional proof of the influence of time in the protein-sparing action of carbohydrates and thus the importance of between-meal feedings of carbo-

hydrates, in order to maintain nitrogen equilibrium in adults and permit optimum storage of protein in children on low-calorie diets. The data include 875 nitrogen determinations of 24-hour samples of urine obtained with diets of different carbohydrate, fat and protein contents divided into three meals a day only and also with diets which included between-meal feedings of different amounts of carbohydrates.

In the interpretation of the following data, it is necessary to point out that, though all of the foods were weighed accurately, their exact content of carbohydrate, fat and protein was not known, because of the variations in chemical composition of food materials. The average values shown in table 1, however, approximate the actual very closely, since they were calculated from a large number of observations. It will be noted that no nitrogen balance was calculated from less than 74 days of observation of diets divided into three meals a day nor upon less than 51 days of observation of diets divided into six meals a day. No balance was, therefore, calculated from less than 200 meals. Furthermore, it should be noted that, this was a comparative study and that there was no reason for greater variations in composition of the food materials in one diet than in another.

All nitrogen determinations of the urines were made in duplicate with the macro-Kjeldahl technic. The combined data are, briefly, summarized in table 1.

It will be noted that, though the excess excretions were not very great, the average values clearly indicate that nitrogen equilibrium was not maintained either with the modified Allen (diet 1) or with the low carbohydrate-high fat-low protein diet (diet 2). The data thus fit in with the earlier experiences of Geyelin and Du Bois ('16). With the moderate amount of carbohydrate—100 gm.—but with no reduction of the fat nor increase of the protein content (diet 3) there was much less difficulty in maintaining nitrogen equilibrium, but the average output of nitrogen still exceeded the average intake. A very definite change of the protein metabolism was, however, noted when the latter diet was replaced by the

TABLE 1

Showing relationship between type of diet and nitrogen balance in diabetes mellitus

	1	2	3	4	5	6	7	8
	MODIFIED ALLEN	LOW CARBOHYDRATE HIGH FAT	MODERATE CARBOHYDRATE HIGH FAT	HIGH CARBOHYDRATE-LOW FAT-MODERATE PROTEIN	SAME AS DIET 4 BUT WITH BETWEEN-MEAL FEEDINGS OF 10 GM. CARBOHYDRATE [1]	HIGH CARBOHYDRATE-LOW FAT-LIBERAL PROTEIN	SAME AS DIET 6 BUT WITH BETWEEN-MEAL FEEDINGS OF 10 GM. CARBOHYDRATE [1]	SAME AS DIET 7 BUT WITH BETWEEN-MEAL FEEDINGS OF 10 GM. CARBOHYDRATE [1]
Diet (average values)								
Carbohydrate	35.4	48.7	108.7	248.7	241.4	256.3	251.4	246.8
Fat	142.8	147.3	142.5	56.0	53.2	44.1	42.9	43.4
Protein	53.1	56.2	52.6	73.1	71.0	104.6	106.7	109.2
Total glucose	80.5	96.0	153.5	296.7	287.9	321.4	317.6	314.4
Total calories	1639	1745	1928	1791	1728	1840	1818	1815
Average weight (kg.)	70.4	68.3	72.5	64.8	70.6	69.8	76.1	74.4
Total protein (gm./kg.)	0.75	0.82	0.72	1.13	1.00	1.49	1.40	1.46
Animal protein								
Total (gm.)	36.3	42.1	40.8	35.8	37.2	66.0	66.9	68.4
Per cent of total	68.4	74.9	77.5	48.9	52.4	63.1	62.7	62.6
Gm./kg.	0.51	0.62	0.56	0.55	0.53	0.94	0.88	0.91
Days of observation	155	74	136	218	111	68	51	62
Nitrogen balance								
Intake	8.5	9.0	8.4	11.7	11.3	16.7	17.1	17.4
Output [2]	10.5	10.7	9.0	10.7	9.7	14.5	13.3	12.7
Retention or loss (gm.)	−2.0	−1.7	−0.6	+1.0	+1.6	+2.2	+3.8	+4.7
Retention or loss (gm./kg.)	−0.028	−0.025	−0.008	+0.015	+0.022	+0.031	+0.050	+0.063
Intake/output × 100	0.81	0.84	0.93	1.09	1.16	1.15	1.28	1.37

[1] The total food value of this diet includes the between-meal feedings.

[2] Includes allowance of 10% of nitrogen in faeces.

high carbohydrate-low fat-moderate protein diet (diet 4); the average intake now exceeded the average output. As was pointed out in the first report of this diet (Rabinowitch, '30) retention of nitrogen is one of its most striking metabolic effects. In fact, subsequent experiences (Rabinowitch, '31) showed that the lowest urinary nitrogen values ever found in man were, judging from the literature (Rabinowitch, '38 a) found with this diet.

The above experiences fit in with the well-known experiments of Kayser and of Tallquist (Sherman, '37) in which, by feeding isodynamic quantities of carbohydrate and fat, it was shown that carbohydrates tend to enhance, whereas fats tend to interfere with, storage of nitrogen in the body. A variable which must, however, be considered in the interpretation of the above data is the higher intake of protein with diet 4 than with diets 1, 2 and 3. It will be noted that, whereas diets 1, 2 and 3 contained about 50 gm. only of protein, diet 4 contained about 75 gm. The difference is, approximately, the same when the intake of protein is expressed as grams per kilogram of body weight. That the increase of protein was an important variable will be noted subsequently.

Diet 5 clearly shows the protein-sparing action of between-meal feedings of carbohydrates with diets of low caloric value. It will be noted that the total allowance of food with this diet was the same as with diet 4; but, from the total allowance, the equivalent of 10 gm. of carbohydrate was fed between meals (11.00 A.M. and 4.00 P.M.) and also before retiring. The result of this change of treatment was a still greater retention of nitrogen.

The origin of these between-meal feedings was entirely accidental, namely, an observation by one of our diabetics that he always felt better whenever he took, from his total allowance, some food (an apple or an orange) between meals. Except for the known fact that frequent feedings prevent the stomach from becoming empty and thus tend to diminish weakness and fatigue (Carlson's accessory phenomena of hunger) the explanation of the improvement of health in

general was not clear until the publication of Haggard and Greenberg on diet and physical efficiency ('35). That these between-meal feedings have approximately the same effect upon the respiratory quotient in diabetics as in non-diabetics was recently shown by one of us (Rabinowitch, '38 b). That much of the improvement subjectively is, however, also due to improvement of the protein metabolism is suggested from comparison of diets 5 and 6. It will be noted that there was a further retention of nitrogen without the between-meal feedings by increasing the intake of protein from about 70 to about 100 gm. a day.

Comparison of diets 6 and 7 shows the value of between-meal feedings even with liberal amounts of protein whenever it is necessary to reduce the caloric content of the diet. It will be noted that though the intake of protein was adequate and the same with both diets (both contained approximately 100 gm. of protein, two-thirds of which was of animal origin) the between-meal feedings of 10 gm. of carbohydrate resulted in a still further retention of nitrogen. Diet 8 shows that a still better result was obtained when the between-meal feedings consisted of 20 gm. of carbohydrate.[1]

Three examples of the combined effects of a) the high level of protein intake, b) the high proportion of animal protein, c) the liberal amount of carbohydrate, d) the small amount of fat, and e) the between-meal feedings are shown in table 2. Children were purposely selected to demonstrate the force of this combination, because of the importance of protein storage for growth and development in general. In the past, as diets 1 to 3 clearly show, nitrogen equilibrium was maintained with very great difficulty in diabetics, though the urines were kept free of sugar and the blood sugars were kept perfectly normal. With hyperglycaemia and glycosuria, storage of nitrogen, judging from the literature and from our own experiences, was a rare phenomenon. The data in table 2

[1] The 20-gm. meal was not accidental; it was based upon the observation by Haggard and Greenberg that 20 gm. of carbohydrate resulted in the maximum increase of net muscular efficiency (personal communication).

are, therefore, significant in that, in spite of the average urinary excretions of 23.6 and 41.0 gm. of sugar per day, the average intake of nitrogen exceeded the average output in two of these cases (6051/32 and 3022/36) by 0.06 and 0.17

TABLE 2

Showing retention of nitrogen in spite of post-prandial glycosuria[1] in severe juvenile diabetics treated with the high carbohydrate-low calorie diet

REMARKS	6051/32	3022/36	5193/38	
Age (yrs.)	6	12	7	
Diet				
Carbohydrate	230	218	210	
Fat	45	42	45	
Protein	72	99	70	
Total available glucose	276	280	255	
Calories	1613	1646	1525	
Average weight (kg.)	20.0	23.1	20.2	
Protein				
a) Total: (gm./kg.)	3.6	4.3	3.5	
b) Animal: Gm.	48.6	72.0	42.0	
Per cent total	67.5	72.7	60.0	
Gm./kg.	2.4	3.1	2.1	
Days of observation	14	12	12[2]	14[3]
Insulin (units/24 hours)	20	45	30.8	35
Urine sugar (gm./24 hours)	23.6	41.0	12.4	0
Nitrogen balance				
Intake (gm.)	11.5	15.8	11.2	11.2
Output (gm.)	10.3	11.8	6.5	7.7
Retention (gm.)	1.2	4.0	4.7	3.5
Retention (gm./kg.)	0.06	0.17	0.23	0.17
Intake/output × 100	1.12	1.34	1.72	1.45

[1] Urine free of sugar and blood sugar normal in fasting state.
[2] Period of glycosuria.
[3] Period of no glycosuria.

gm. respectively per kilogram of body weight. That these children were not mild diabetics is clearly shown by comparison of their ages with their insulin requirements.

With diets of total available glucose values of 276 and 280 gm., average excretions of 23.6 and 41.0 gm. of sugar are not very great losses and, as was shown in another report,[2] differ-

[2] Rabinowitch, I. M. The significance of post-prandial glycosuria. Ann. Int. Med. In press.

ing from the experiences with low-carbohydrate-high fat diets of the past, post-prandial glycosuria is not harmful with the high carbohydrate-low fat diet, providing the blood sugar is normal in the fasting state. Glycosuria is, however, harmful when there is hyperglycaemia in the fasting state. The data of the third child (5193/38) are, therefore, instructive in that storage of protein occurred in spite of the poor control of the diabetes, as shown by the degree of hyperglycaemia in the fasting state. It will be noted that when the data were divided into two periods, namely, a) when the urine was free of sugar and b) when the urine contained sugar, there was no greater—in fact there was less—retention of nitrogen during the sugar-free period than when the urine contained sugar. The maximum, minimum and average blood sugars in the fasting state, in the corresponding periods, were 0.277, 0.217, 0.243 and 0.250, 0.175 and 0.204% respectively. In view of the experiences with the old high fat-low carbohydrate-low protein diets, the explanation of the storage of the protein appears to be enhancement of the protein-sparing action of the carbohydrates by the between-meal feedings, combined with the influence of the high carbohydrate-low fat intake and the liberal supply of protein, quantitatively and qualitatively.

The above nitrogen balances fit in with our experiences with skeletal growth in juvenile diabetics. Two examples are cited in figures 1 and 2. Figure 1 is that of a girl age 12 years, 8 months (1528/35) who, prior to her admission to this clinic, had been treated for the diabetes by drastic reduction of food. According to the description of her diet she received approximately 1.5 gm. of protein per kilogram of body weight and the protein of animal origin accounted for approximately 1.1 gm. per kilogram. It will be noted that she was markedly underheight, according to her age (M. S. Rose, '38)—the actual and expected heights were 48.0 and 55.5 inches respectively. Shortly after the carbohydrate content of the diet was increased and the total protein was increased to 3.0 gm., and the animal protein to 2.2 gm., per kilogram, she

began to grow rapidly and, at the end of about 3 years, the actual height was within about 2 inches of the expected (she gained 9½ inches in 35 months) whereas, prior to the change of diet it was, as stated approximately, 7½ inches below that expected for her age.

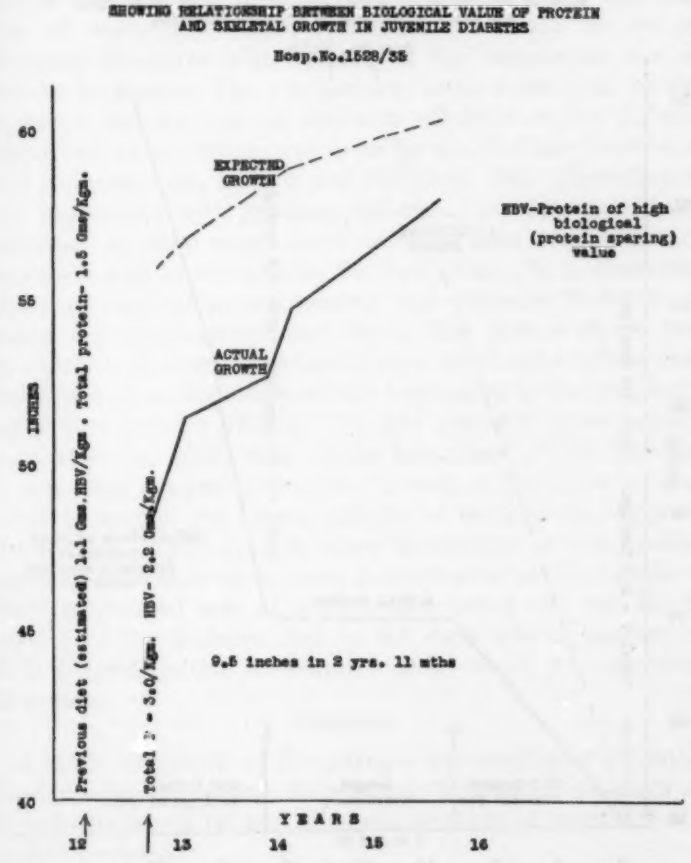

Figure 1

Figure 2 is that of boy (70/28) who, at the age of 7 years, was normal with respect to height. The total protein content of the diet appeared to be adequate, but it will be noted that, until the age of about 9½ years, he failed to grow at the normally-expected rate and, from 9½ to 12½ years, the height

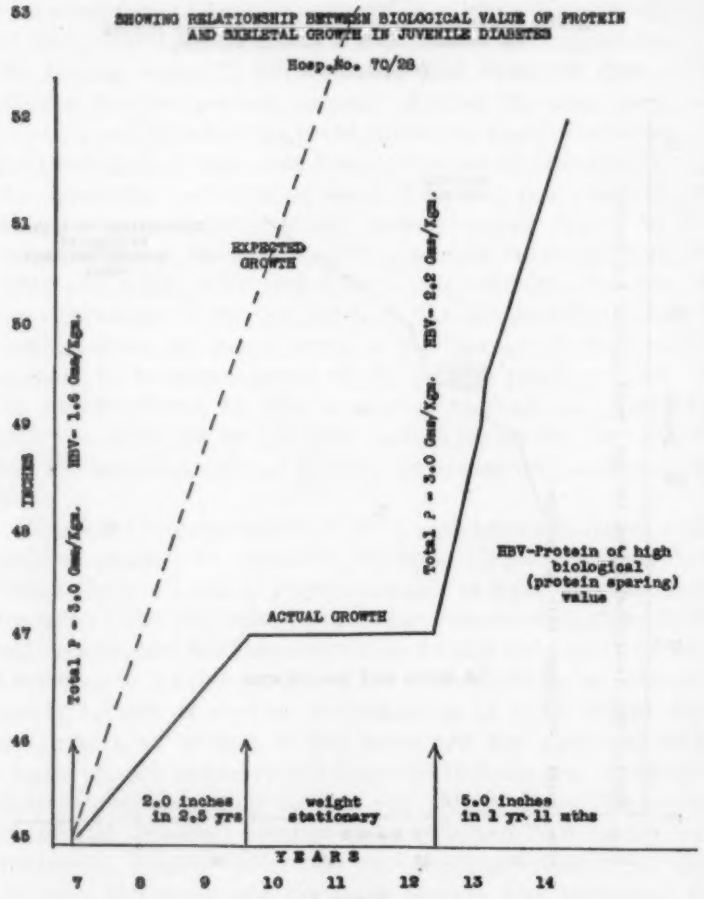

Figure 2

remained stationary. The animal protein was then increased from 1.6 to 2.2 gm. per kilogram of body weight. Thereafter, he began to grow at a very rapid rate and gained 5 inches during the next 23 months.

In view of these experiences with skeletal growth, it is appropriate to emphasize the importance of the above-mentioned variables which should be considered in the construction of low-calorie diets, particularly because of the increasing literature with respect to the therapeutic use of growth hormones. There appears to be no doubt that growth hormone therapy may at times be effective in the diabetic (Beck and Suter, '38) as well as in the non-diabetic (Lawrence and Harrison, '38; Molitch and Poliakoff, '38). According to our experience with juvenile diabetes, however, nutritional dwarfism is very much more common than true pituitary dwarfism and is correctable by diet alone. In a statistical study of skeletal growth among our juvenile diabetics 10 years ago (Rabinowitch and Bazin, '29) it was shown that though the children, in general, were overheight before they developed their diabetes and thus conformed to the generally-recognized pattern (White, '27) they ceased to grow rapidly soon after the institution of the treatment of the diabetes. It was then suggested that the control of the diabetes also tends to control the excess activity of the growth hormone. Since then, however, with more knowledge of the protein metabolism in such cases, there is much more reason to believe that the reduced rate of growth then noted was not due to control of the diabetes, but to the diets which, because of their deficiencies, did not permit storage of adequate amounts of protein.

SUMMARY

A study was made of the nitrogen metabolism of diabetics treated with low calorie diets but with different amounts of carbohydrate and fat and different amounts of vegetable and animal proteins.

The findings fit in with previous observations that carbohydrates tend to enhance, whereas fats tend to interfere with

storage of protein in the body; and that the latter is not only also influenced by the total amount of protein in the diet, but also by the relative amount of protein of high biological (protein-sparing) value.

The observation made with diets of normal caloric content that time is a factor which influences the protein-sparing action of carbohydrates is confirmed with diets of low caloric contents.

The many practical applications of these newly-established facts of nutrition are suggested from the many people who for different reasons in health or disease must reduce the caloric contents of their diets. Experiences with skeletal growth in diabetic children are cited as examples.

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A NEW LOW FLUORINE DIET AND ITS EFFECT UPON THE RAT [1, 2]

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Attempts have been made in several laboratories to produce fluorine-free diets. Sharpless and McCollum ('33) prepared a highly purified diet which they state "was very low in fluorine but not quite free." Marcovitch, Shuey and Stanley ('37) used rice and milk to obtain a ration which contained about 0.6 part of fluorine per million. Fellenberg ('37) and Tamman (1888) have reported traces of fluorine in milk. Repeated analyses of milk made in this laboratory have shown that it contains between 0.1 and 0.2 parts of fluorine per million (Phillips, Hart and Bohstedt, '34). Since milk can be converted to an adequate diet by mineralization it would seem that mineralized milk would offer an excellent means of studying the effects of a low fluorine diet upon the rat.

EXPERIMENTAL

These experiments were designed to study the effect of this low fluorine diet upon the skeletal fluorine, growth and reproduction in the rat. Fresh milk was mineralized with 1 mg. of iron ($Fe_2(SO_4)_2$), 0.1 mg. of copper ($CuSO_4$) and 0.1 mg. of manganese ($MnSO_4$) per 100 cc. Young rats weighing

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[3] Moorman Manufacturing Company fellow.

40 gm. each were divided, one male and three females per lot, as follows:

Lot	
I	Basal ration—mineralized milk
II	Basal + 0.1 p.p.m. fluorine as NaF.
III	Basal + 1.0 p.p.m. fluorine as NaF.
IV	Basal + 10.0 p.p.m. fluorine as NaF.
V	Basal + 20.0 p.p.m. fluorine as NaF.
VI	Basal + 20.0 p.p.m. aluminum as $AlCl_3$.
VII	Basal + 20.0 aluminum as $AlCl_3$ + 20.0 p.p.m. fluorine as NaF.
VIII	Basal + 20.0 p.p.m. fluorine as NaF + 3 drops of percomorph oil per rat per week.

In this experiment an attempt was made to determine if aluminum or excess vitamin A would reduce the effects of the higher levels of fluorine. It was planned that the animals on experiment would be raised to maturity and allowed to reproduce. The animals were kept on raised wire screens except when pregnant females were ready to litter. In the latter case the females were put on shavings. All attempts to raise litters on screen floors failed. At birth the litters were reduced to four pups. The remaining pups were taken immediately for fluorine analyses. At 21 days one young rat from each lot was likewise taken for analyses to determine if a large increase in fluorine concentration occurred during the suckling period. The remaining young were used for the production of a second generation. A third and fourth generation were likewise obtained. The adult rats were removed when the succeeding generation rats were produced. Some remained on experiment 4 and some 7 months. In these cases the tibia and femurs were analyzed to determine the skeletal fluorine content.

The fluorine analyses were made by the modified method of Willard and Winter as previously described (Evans and Phillips, '38).

RESULTS

Mineralized milk proved to be a good low fluorine ration. On this diet the rat remained healthy and vigorous through

five generations. Food consumption records showed that only 0.05 to 0.06 mg. of fluorine per kilogram of body weight were ingested per day. It is plainly evident that in this species, which can on occasion withstand relatively large quantities of fluorine, very little fluorine is necessary.

In these experiments the growth records of the young were alike. There was no retardation of growth when 20 parts of fluorine per million were fed. Neither did the added aluminum or percomorph oil affect growth. On the basal ration plus 20 p.p.m. of fluorine the incisor teeth were definitely bleached and elongated. Food consumption indicates that this occurred on an intake of 5 mg. of fluorine per kilogram of body weight per day. The marginal level where bleaching was first noticed was found to be 10 p.p.m. At this level the rats ingested 2.5 to 3.0 mg. of fluorine per kilogram of body weight per day. Above 3.0 mg. of fluorine per kilogram of body weight per day bleaching always occurred. Below this level the teeth were normal in appearance. No beneficial results attended the addition of 0.1 or 1.0 part of fluorine per million to the milk. Neither aluminum at the level fed here nor the percomorph oil prevented bleaching of the incisors.

Reproduction difficulties were observed during mid- and late winter. This explains why the second generation required 7 months to produce the third generation. In general the levels of fluorine administered had no beneficial or adverse effect upon reproduction. Failure occurred in lot V but previous experience had shown us that rats on this ration would reproduce for three consecutive generations. Lots VI and VII also failed to reproduce beyond the first generation.

Table 1 shows the average fluorine content of the young rats by generations. It is evident that the newborn in lot I on the basal ration alone contained appreciable fluorine. This residual fluorine was not greatly affected until the added fluorine reached 10 or more parts per million, and then there seemed to be a rather distinct increase in the first generation except in one case (lot VIII). There was no significant difference between the fluorine content of the young of the first

and third generations on the respective levels of fluorine fed. There seemed to be no cumulative effect carried over from generation to generation and this applies to both the lowest and the highest levels administered. Unborn fetuses gave similar fluorine contents to the day-old young. This indicates that the young at birth were not greatly contaminated with fluorine.

It has been estimated from our analytical data that 33% of the basal ration fluorine ingested during the gestation period was deposited in the fetus. The actual dry matter represented in the fetuses was only 5.1% of the total solids

TABLE 1

The average fluorine content of young rats (fluorine in p.p.m.)

	LOT I BASAL	LOT II BASAL + 0.1 ppm F	LOT III BASAL + 1.0 ppm F	LOT IV BASAL + 10 ppm F	LOT V BASAL + 20 ppm F	LOT VI BASAL + 20 ppm Al	LOT VII BASAL + 20 ppm F + 20 ppm Al	LOT VIII BASAL + 20 ppm F + percomorph oil
At birth								
F1	12.0	8.5	15.6	19.2	19.2	7.0	23.6	6.7
F2	..	8.8	..	24.5	..	..	..	47.7
F3	10.6	10.3	11.3	21.4	..	..	..	19.0
At 21 days								
F1	5.7	8.4	7.8	25.0	7.2	6.0	5.9	5.2
F2	4.1	4.0	..	24.6	..	..	..	24.6
F3	3.5	5.3	6.2	22.0	..	..	..	46.7

ingested. Thus the residual fluorine in the fetus seems to be larger than one would expect from the fluorine content of the ration. This suggests a preferential storage of the milk fluorine in the fetal tissues. This suggestion is further supported by the fact that females which reproduced contained smaller amounts of skeletal fluorine than their respective lot mates. These data show a definite placental transfer of fluorine.

It is also seen from table 1 that there was comparatively little storage of fluorine during the suckling period. The fluorine increase from birth to 3 weeks was slightly more than doubled. The dry matter of the rat at birth averaged 1.0 gm.

and contained 10 μg. of fluorine. At 21 days the dry matter averaged 5.0 gm. and the fluorine content 22.5 μg. Thus the proportion of fluorine stored in the rat in comparison to the increase in body weight was considerably less during the suckling period. There was no increase in fluorine storage during the suckling period when the mother received as much as 20 p.p.m. of added fluorine.

Table 2 shows the relative skeletal storage of fluorine with respect to the fluorine ingested by the various lots. On the basal ration alone the average skeletal fluorine was 8 to 16 p.p.m. irrespective of the length of time on experiment. The addition of an equivalent amount of fluorine as NaF to that

TABLE 2

The average skeletal fluorine content of the adult rats (fluorine in p.p.m.)

GENERATION	AGE MONTHS	LOT I BASAL	LOT II BASAL + 0.1 ppm F	LOT III BASAL + 1.0 ppm F	LOT IV BASAL + 10 ppm F	LOT V BASAL + 20 ppm F	LOT VI BASAL + 20 ppm Al	LOT VII BASAL + 20 ppm F + 20 ppm Al	LOT VIII BASAL + 20 ppm F + percomorph oil
F	4	16	72	297	1639	2562	71	2339	2335
F1	7	16	44	355	2781	4522	42	4067	4527
F2	4	16	54	265	1729	..	..	..	3065
F2	7	8	54	341	2695	..	..	..	4063
F3	4	10	71	265	1573	..	..	..	2992
F intake mg./kilo/day		0.05–0.06	0.075–0.082	0.30–0.42	2.5–3.2	5.05	0.05	5.05	4.9–6.3

already present in the basal ration resulted in more than a threefold increase in the skeletal storage. When the added fluorine was increased to 1.0 p.p.m., however, the portion stored was distinctly not linear. It appears that the fluorine naturally occurring in the milk is less readily available for storage in the skeleton. Again there seem to have been no significant changes in the skeletal fluorine from generation to generation regardless of the dietary level fed. Certainly the third and fourth generation rats on this low fluorine diet had bones similar in fluorine content and gross appearance to those of the first. These observations again point to the fact

that this ration was adequate in its fluorine content for the rat.

When 1.0 or more parts of fluorine per million were fed, there was a greater storage of fluorine in the skeleton during a 7-month period than there was during a 4-month period. Twenty parts of aluminum per million gave a slight protective action against the storage of fluorine in the skeleton and this protective action was more noticeable with the increase in the length of the experimental period. This level of aluminum did not protect against bleaching of the teeth. Higher levels of aluminum (100 p.p.m.) on this diet do protect over a 2-month period. Aluminum added to the basal diet did not prevent the fluorine present in the milk from being stored in the skeleton even though the ratio of aluminum to fluorine reached the figure of 40:1. Vitamin A in these experiments was not efficacious in reducing the fluorine deposited in the skeleton.

DISCUSSION

The data presented here indicate that excellent results have been obtained using milk as a low fluorine diet. On the basis of dry matter this ration contained 1.6 p.p.m. The skeletal fluorine averaged 13 p.p.m. with a range from 1 to 31 p.p.m. Sharpless and McCollum ('33) obtained skeletal fluorine contents varying from 6 to 28 p.p.m. with an average of 21 p.p.m. on their diet. Marcovitch, Shuey and Stanley ('37) obtained an average of 36 p.p.m. in the skeletons of their rats which were on experiment for a shorter length of time than those in these experiments.

These results show that fluorine is transferred through the placenta. This agrees with our results obtained with cattle (Evans, Phillips and Hart, '38). Whether these results indicate that fluorine is necessary for the developing young is not known. Previous work (Evans, Phillips and Hart, '38) has shown that extraordinary amounts of fluorine are present in embryonic cartilage ash and the organic matrix of bones.

The physical appearance of the bones from the animals fed only the basal ration appeared to be normal in every respect.

The bones were strong, smooth, evenly and well calcified. It appears that the low fluorine found in milk does not produce an inferior bone.

SUMMARY AND CONCLUSIONS

Mineralized milk proved to be a good low fluorine diet through five generations of rats. Summer milk properly mineralized supported reproduction while winter milk was inferior in this respect.

Fluorine is not necessary for the rat in amounts larger than the 0.1 to 0.2 p.p.m. found in milk. Thus 50 μg. of fluorine per kilogram of body weight per day meets all of the requirements of the rat for growth, reproduction and general well being. Additional fluorine from 0.1 to 20.0 p.p.m. caused no measureable improvement in the rat.

The borderline zone for bleaching of the teeth occurred at a level of 10 p.p.m. Bleaching was not prevented by aluminum or the extra vitamin A furnished by percomorph oil used in these experiments.

There was a placental transfer of fluorine even on the low level of fluorine ingested. This was not increased until the level of fluorine reached 10 p.p.m. Thereafter there was a definite increase in fluorine passing through the placenta. Mammary secretion of fluorine, on the other hand, was not affected by as much as 20 p.p.m.

The low level of fluorine furnished by the basal milk diet did not deplete the fluorine stores of the rat through five generations, nor did it increase the demand for fluorine. There was no cumulative fluorine effect carried over from generation to generation on either the lowest or the highest level.

The fluorine normally present in milk appeared to have been less readily metabolized for storage in the skeleton than that added as sodium fluoride.

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INDIAN AND ESKIMO METABOLISMS

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During the summer of 1937 members of the Cleveland Clinic Expedition to northeastern Canada and the subarctic regions of Churchill Bay and Chesterfield Inlet made a series of metabolism studies on thirteen Chippewa Indians living on a reservation near Churchill and on sixty-three Eskimos living in the vicinity of Chesterfield Inlet. Six of the Indians were males and seven were females; of the Eskimos, thirty were males and thirty-three were females. A number of the tests included here were made by Dr. Thomas Melling, Medical Health Officer for the District of Keewatin, Canada, who has his headquarters at Chesterfield. After having been instructed in the use of our Jones metabolism apparatus he carried on many of the tests on the Eskimos.

In each instance a short preliminary test was made with the individual undergoing the test presumably under basal conditions. Following this, the next test was recorded and it is this record in every case which is represented in our metabolism data. The individuals tested were instructed to eat no food following their evening meal on the day prior to the test. From the appearance of the Indians' tents on the morning of the test plus their apparent willingness to cooperate with us induced in part, perhaps, by the promise of small presents, we believe that they obeyed this injunction against taking food. Doctor Melling followed this same general procedure with the Eskimo subjects and found no difficulty in securing their cooperation. Besides this, he is well known to them and enjoys their confidence. Since the matter of having

meals at a set time is of no great moment to either Indian or Eskimo, this request to forego breakfast combined with an admonition to rest prior to the test, involved no hardship for the subjects of the test.

BASAL METABOLISM TESTS ON CHIPPEWA INDIANS

The tests upon the Chippewa Indians were carried out on warm, sunshiny days in early August. The tests were made upon a wide bench in the open, but sheltered from the wind. Before the test the individuals reclined in their tents and remained so until called for the tests.

A word might be said with reference to the habits of the Chippewa Indian in this region. He lives in tents the year around, he does a little hunting and fishing and counts on the Canadian Government for support in the event that his own efforts fail to provide him with sufficient food and clothing to maintain himself. Apparently he makes less effort to fend against the inhospitable climate than does the Eskimo, and he does not show the same adaption to the rigors of northern life as does the Eskimo. His food consists of fish and game, eked out with 'store' food where he can procure it.

Table 1 sets forth the data as secured on the Indian males and females. Included in these data are age, weight, height, as well as blood pressure, temperature, pulse rate and metabolism percentage based upon the Mayo normal standard records as established for white individuals of corresponding age, sex and body size.

METABOLISM

Disregarding the values over +40 in the male as well as in the females, the average metabolic values are +18% for the male Chippewa Indian and +18.50% for the female Chippewa Indians. These values we believe to be approximate basal values for this group.

PULSE AND BLOOD PRESSURE

We were surprised at the slow pulse rate in the Indian. This ran significantly lower than did the pulse rate of the

Eskimo. The average pulse rate for the Indian males was 53.3 against 70 for the male Eskimo and 63 for thirty-five Maya-Quiché Indian males whose records were secured in Guatemala. In the female Indian the average pulse rate was 65 as compared with 78.8 for the Eskimo female and 82 for five

TABLE 1

Chippewa Indians

Males

NUMBER	AGE	HEIGHT	WEIGHT	TEMPERATURE	BLOOD PRESSURE		PULSE	O_2 CONSUMED PER MINUTE	DEVIATION FROM MAYO NORMAL STANDARD
	Years	*cm.*	*kg.*	*F.*	*Sys.*	*Diast.*		*cc.*	%
1	21	163.8	61.2	96.8	112	78	55	258	12[1]
2	40	176.9	70.3	97.0	102	75	42	...	..
3	33	170.8	60.3	97.0	128	85	48	250	9
4	28	166.4	60.3	96.5	118	78	56	266	16
5	24	166.4	63.0	96.3	134	80	62	320	35
6	30	168.9	65.8	95.8	126	85	58	333	44
Av.	29	168.8	63.4	96.9	120	80	53.3	273	+18.0 without no. 6
				Female	Indians				
7	15	157.5	48.5	98.0	122	74	52	216	5
8	17	158.7	54.9	97.8	116	72	62	333	64
9	50	166.4	68.5	97.2	120	70	72	253	23
10	33	160.0	60.3	97.4	145	86	60	266	39
11	50	151.8	61.7	98.4	114	72	78	210	8
12	30	152.3	58.9	97.0	128	78	54	235	22
13	33	157.4	66.2	97.4	126	76	78	235	14
Av.	33	157.7	59.8	97.6	124	75	65	236	+18.5 without no. 8

[1] All values in final column are 'plus' unless otherwise indicated.

Maya-Quiché Indian females. The pulse of these northern Indians is characterized by the fullness, and regularity of the beat.

The blood pressure of the Chippewa Indians averaged 120/80 for the males and 124/75 in the females as against 119/75

for the Eskimo males and 112/72 in the Eskimo female compared with 111/77 for thirty Maya-Quiché Indian males and 111/75 for five Maya-Quiché females in a series of tests made in Guatemala, C. A.

Considering the nomadic habits of these northern Chippewa Indians, their extreme endurance, which in many instances rivals or exceeds that of the Eskimo in the sense that they face the extreme rigors of the climate and yet fail to provide the warm shelters for themselves as do the Eskimos, and by their failure to clothe themselves as warmly as do the Eskimos either in summer or in winter, the relatively high metabolic rate might be explicable on these grounds. Considering the effect of environmental temperature upon the basal metabolic rate, any continued exposure to the prevailing temperatures at the latitude of Churchill, which marks the northern limit of the Indian, might be expected to raise this rate significantly. On the other hand, the Maya-Quiché Indian of Central America according to our own findings and those of Steggerda, Williams, Shattuck, Benedict, the Navajo Indian of Arizona according to Salsbury, all tend to show a basal metabolic rate significantly higher than the standard estimates for whites of similar ages and weights. It is evident that this higher basal rate is not a Mongolid characteristic since the studies of MacLeod, Crofts and Benedict, Knipping, Takahira, all show the southern Mongolid types (the Sinid and the Palaeomongolid) run a lower basal metabolic rate than do the Indians or the Eskimos measured on this continent which presumably are an offshoot of the North mongolid or Tungid type of the human race.

BASAL METABOLISM TESTS ON ESKIMOS

The Eskimos represented in this study live almost entirely on a native diet according to Doctor Melling. Their food consists of caribou, seal, walrus, fish and birds. They still live in caribou skin or canvas tents in summer and snow huts in the winter.

Table 2 represents the data secured from thirty male Eskimos between the ages of 15 and 85 years with an average age of 36.8 years. The average nude weight of these Eskimos was 64.9 kg., or approximately the same as prevails for male whites in the United States. The average male height of 5 feet 5 inches is somewhat under the white average.

TABLE 2

Eskimo males

NUMBER	AGE	HEIGHT	WEIGHT	TEMPERATURE	BLOOD PRESSURE		PULSE		O_2 CONSUMED PER MINUTE	DEVIATION FROM MAYO NORMAL STANDARD
	Years	*cm.*	*kg.*	*F.*	*Sys.*	*Dias.*	*Bef.*	*Aft.*	*cc.*	%
14	47	165.1	72.5	97.4	118	86	54	66	250	8[1]
15	85	161.3	57.6	97.0	92	70	67	84	206	8
16	40	170.2	72.5	97.0	138	80	72	60	308	27
17	18	155.0	75.2	96.4	122	78	66	84	286	24
18	22	170.2	63.9	98.0	148	80	72	84	286	17
19	28	155.0	58.1	98.3	116	72	64	62	235	9
20	30	160.0	67.1	96.6	124	70	64	70	242	5
21	37	157.4	66.7	96.3	162	90	62	68	286	29
22	40	157.4	58.1	96.3	108	64	60	60	235	13
23	70	172.1	73.5	96.3	112	70	68	64	250	11
24	50	158.1	62.1	96.1	122	70	80	78	285	35
25	65	167.7	64.4	96.0	120	60	78	72	235	13
26	15	153.7	48.5	96.3	120	65	72	80	242	4
27	30	158.8	50.8	97.4	112	78	60	56	266	18
28	64	165.1	75.7	97.0	120	84	66	60	333	42
29	50	165.1	77.1	96.0	114	78	60	68	258	9
30	55	161.3	61.7	96.2	138	98	80	76	258	21
31	22	157.7	58.9	98.1	102	68	72	72	242	8
32	24	163.8	63.0	98.0	104	68	80	80	273	16
33	45	175.2	78.0	97.3	116	78	72	56	286	14
34	55	165.1	72.1	96.4	102	68	60	56	250	12
35	25	159.4	64.4	97.6	138	86	60	60	273	18
36	35	171.5	72.1	96.0	118	84	76	60	266	7
37	28	166.3	75.3	97.1	126	88	76	76	286	14
38	40	158.1	58.5	97.1	128	78	72	72	250	21
39	18	157.5	46.3	97.3	96	78	80	80	242	18
40	18	160.7	54.9	96.8	116	62	64	64	250	8
41	38	160.0	71.2	96.6	108	60	56	64	286	23
42	38	171.5	78.5	96.0	122	78	60	60	363	42
43	18	160.0	60.3	96.0	102	64	72	72	235	−3
Av.	38.3	162.7	65.3	96.8	118.8	75.1	61.9	68.8	265.3	+14.5 without +42 values; +16.4 including all values

[1] All values in final column are 'plus' unless otherwise indicated.

TABLE 3

Eskimo females

NUMBER	AGE	HEIGHT	WEIGHT	TEMPERATURE	BLOOD PRESSURE		PULSE		O_2 CONSUMED PER MINUTE	DEVIATION FROM MAYO NORMAL STANDARD
	Years	*cm.*	*kg.*	*F.*	*Sys.*	*Dias.*	*Bef.*	*Aft.*	*cc.*	%
44	47	152.4	58.9	97.0	124	80	66	66	266	42[1]
45	18	148.5	51.3	98.0	122	78	90	84	235	29
46	16	147.9	53.1	98.0	126	80	72	90	286	45
47	21	151.1	55.8	98.1	128	78	72	78	258	38
48	42	153.7	56.6	98.3	98	68	96	84	258	43
49	56	154.9	57.2	97.2	124	72	84	66	222	26
50	17	144.7	45.8	97.2	104	68	60	72	216	23
51	16	138.4	44.4	98.5	114	72	58	74	308	76
52	38	149.8	55.3	97.5	125	78	70	80	205	12
53	50	152.4	61.0	96.1	120	68	76	68	250	35
54	45	150.4	55.3	97.3	108	70	80	76	195	8
55	45	158.1	54.0	95.5	118	78	80	76	205	11
56	16	154.9	52.2	97.3	108	68	92	96	222	10
57	35	153.0	54.0	96.1	136	80	84	92	258	40
58	18	161.3	46.7	97.0	102	70	88	84	222	19
59	22	156.2	66.7	97.0	124	88	84	84	258	25
60	55	151.1	53.1	97.0	130	86	80	84	222	31
61	45	140.9	51.3	98.1	138	80	76	84	235	40
62	40	151.1	58.9	98.1	108	74	64	76	286	51
63	20	148.5	53.1	97.0	108	64	76	80	222	23
64	18	158.1	59.4	97.0	108	68	84	80	206	0
65	45	143.5	50.3	97.0	112	66	64	76	266	58
66	18	157.3	67.0	97.4	116	70	76	88	235	9
67	27	146.7	55.8	96.2	110	72	80	76	222	23
68	35	152.4	61.2	98.3	110	70	100	104	242	23
69	45	160.6	57.6	98.0	88	68	68	68	222	20
70	22	163.2	58.5	96.4	90	70	76	80	348	70
71	16	151.1	48.5	97.8	96	68	92	92	216	13
72	19	147.0	59.9	97.0	102	58	64	52	222	15
73	30	151.1	49.0	96.3	98	64	64	84	222	26
74	40	152.4	54.1	98.6	104	68	92	96	235	29
75	20	148.5	55.3	98.1	118	64	96	116	235	27
76	40	147.9	56.2	97.2	94	60	108	100	242	31
Av.	31.4	151.5	55.1	97.3	112.1	71.7	78.8	82.4	240.5	+21.12 without 40 or over values; +29.4 including all values

[1] All values in final column are 'plus' unless otherwise indicated.

The average basal metabolic rate for these male Eskimos excluding values over +40 was +14.5. Including all values the average was +16.4.

Table 3 shows the data secured from thirty-three female Eskimos. The age range in this group was from 16 to 65 years with an average age of 32.4 years. The average nude weight of the group was 55 kg. or just about the average for American female whites.

The average metabolic rate recorded for the entire group of female Eskimos was +29.4. Omitting all values of +40 or over the average values of the remaining 24 individuals was +21.12.

TABLE 4

GROUP MALE AND FEMALE	MEAN METABOLISM PER CENT + B. M. R.	STANDARD DEVIATION	COEFFICIENT OF VARIATION PER CENT
Male and female Indians	18.25±3.46	10.90±2.55	58.8±10.31
Male Eskimos	14.50±1.62	8.50±1.13	58.46± 7.81
Female Eskimos	21.12±1.76	8.65±1.25	38.41± 5.54

PULSE AND BLOOD PRESSURE

The pulse rate of the male Eskimo, 63 to 69 as well as that of the female at 79 to 83 falls perhaps slightly under the pulse values of white males and females and is markedly higher than that of the Chippewa Indians.

The average blood pressure for the males was 119 systolic, 75 diastolic and for the females it ran 112 systolic and 71 diastolic. The average height of 5 feet ½ inch appears to be considerably lower than the white female average.

SUMMARY

Metabolism tests were made on six male and seven female Chippewa Indians. The average metabolic values recorded for the males was +18.0% for the females it was +18.5%. Pulse rate and blood pressure both appear to be lower than they are for the American white population or the Eskimo, or Mayan Indian of Central America.

The metabolism tests run on thirty male Eskimos from the region around Chesterfield Inlet, Canada, gave an average reading of +16.4% for the males and +29.0% for thirty-three females when the values above +40 are included; without the values above +40% the averages for the males are +14.5% and for the females they are +21.12%. The blood pressure for both the males and the females is lower than that of whites of corresponding age, the pulse rate corresponds rather closely to that of white individuals.

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A STUDY OF THE METABOLISM OF THE MAYA QUICHÉ INDIAN

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During a recent expedition to Guatemala, Central America, 164 basal metabolism estimations were made on thirty-five male Maya Quiché Indians. Thirty of the subjects were soldiers with from 6 months to a year of army service. These men were stationed at Totonicipan at an altitude of 8100 feet. Five were coffee plantation laborers stationed on the Pacific slope at an altitude of 800 feet. In addition, eight tests were made upon one white male and one white female at 8100 feet and on one white male living at 800 feet altitude. All the tests were run in December, 1938, and January, 1939. The study on the soldiers was made possible through the courtesy and the assistance of the governor of Totonicipan Province, Colonel Carlos Cipriani.

To test the possible stimulatory effect of chili, twenty tests were run on ten soldiers 3 hours after they had ingested measured amounts of chili. As a check on the chili tests six subjects were tested 3 hours after eating from 1 to 10 gm. of tortillas (corn cakes). From four to eight tests were made upon each subject in the course of a week. The usual data necessary for making metabolism determinations were taken. The soldiers were tested in their barracks and after the initial test took little interest in the proceedings commonly falling asleep while waiting their turn to be tested. Following the evening meal on the night before the test, they remained in their bunks until the conclusion of the tests. During the tests

TABLE 1

Maya-Quiché metabolism data

NUMBER	AGE	HEIGHT	WEIGHT	TEMPERATURE	BLOOD PRESSURE		PULSE		NUMBER OF TESTS	O_2 CONSUMED PER MINUTE	DEVIATION FROM MAYO NORMAL STANDARD	PERCENTAGE CHANGE OVER SUBJECT'S OWN BASAL RATE AFTER CHILI FEEDING	PERCENTAGE CHANGE OVER SUBJECT'S OWN BASAL RATE AFTER TORTILLA FEEDING	DOSAGE OF CHILI OR TORTILLA
	years	*cm.*	*kg.*	*F.*	*Sys.*	*Dias.*	*Bef.*	*Aft.*		*cc.*	%	%	%	
1	22	154	54.0	98.6	111	79	61	64	7	242	+13	+10.8		1 gm. dry chili
2	18	154	50.1	97.5	108	77	58	60	6	232	+7			
3	26	161	62.5	98.0	105	79	80	86	5	258	+12			
4	22	154	55.2	97.5	128	85	56	48	7	222	+5		−2.86	10 gm. tortilla
5	19	159	57.1	97.6	105	81	64	56	7	240	+5	+11.5		1 gm. dry chili
6	22	157	54.9	97.8	108	77	64	48	6	222	+6			
7	19	162	61.5	97.2	122	85	56	54	7	244	+6	+13.3		11 gm. liquid chili = 3 gm. dry
8	22	158	56.1	97.0	108	90	58	52	8	243	+14		0.0	10 gm. tortilla
9	24	172	69.5	98.3	108	74	60	64	7	251	+2	+12.7		12.18 gm. liquid = 3 gm. dry
10	23	165	57.3	97.2	108	75	66	66	8	243	+7	+14.0		12.53 gm. liquid = 3 gm. dry
11	27	168	64.0	97.0	112	75	60	54	6	260	+10	+11.0		11.20 gm. liquid and corn = 3 gm. dry
12	21	166	69.0	98.0	100	73	66	60	4	280	+13			
13	22	152	53.9	98.6	118	85	90	96	4	243	+14			
14	19	157	62.0	98.4	128	77	62	62	4	235	0			
15	24	155	54.9	97.5	115	85	54	54	4	222	+6			
16	22	159	57.1	97.6	112	79	54	54	6	219	−1		0.0	10 gm. tortilla
17	24	155	55.2	97.2	108	65	48	52	6	236	+11		+10.8	10 gm. tortilla
18	20	161	57.1	97.6	110	70	68	64	4	234	+5			
19	20	160	64.0	97.8	115	71	66	68	6	254	+10	0.0		1 gm. dry chili
20	21	165	61.5	97.6	124	76	60	62	6	242	+3	10.6		1 gm. dry chili

21	24	168	65.0	98.0	108	75	60	66	4	237	0			
22	18	157	54.9	97.5	105	71	66	66	4	242	+11			
23	25	156	54.0	98.0	110	67	54	54	4	240	+13			
24	24	158	58.0	98.0	115	75	56	56	4	232	+7			
25	24	156	54.1	97.6	118	77	48	48	4	222	+5			
26	22	153	52.0	97.8	112	75	48	52	4	211	+1			
27	33	158	59.4	97.6	115	80	66	66	6	264	+22		−13.2	10 gm. tortilla
28	19	156	57.6	97.2	110	79	54	56	6	219	−1		+13.1	10 gm. tortilla
29	19	156	58.0	97.0	116	73	72	66	6	245	+8	+1.018		1 gm. dry chili
30	26	158	53.2	98.0	98	75	72	72	6	239	+16	−2.6		1 gm. dry chili
Average:														
	22.39	159.3	57.57	97.6	111	77	63	61	166		+8.20	+8.46	+1.30	
Coffee plantation laborers														
31M	20	149	42.0	97.4	105	75	60	57	6	212	+12			
33	23	167	52.1	97.5	108	79	66	63	6	243	+10			
35	19	167	48.0	97.5	95	70	64	61	6	229	+6			
37	22	165	50.1	97.4	114	73	60	58	6	200	−6			
39	24	159	57.1	97.4	108	68	62	63	6	228	+4			
Average:														
	21.6	161	49.8	97.4	104	73	62	60	30		+5.2			
White persons														
N.R(F.)	34	163	62.6	97.5	116	77	72	72	2	194	−6			
A.G(M.)	36	171	70.5	97.5	135	77	72	68	2	210	−13			
J.M(M.)	25	175	67.5	96.7	122	69	72	72	4	222	−12			
Average on two males:														
	30.5	173	69.0	97.1	128.5	73	72	70	8		−12.5			

[1] Unless indicated with a minus sign, all values are plus.

they were under constant observation. A Jones metabolism [1] unit provided with an oversized bellows and adapted for field work was used in this study.

DISCUSSION OF RESULTS

Tables 1 and 2 set forth the data secured and the mean metabolism rates respectively. The tests ranged from a −1 to +22% which represents the mean calculated percentage based upon the Mayo normal standard established for white

TABLE 2

Mean metabolism rates

LOCATION	NUMBER OF TESTS	SEX	NATURE OF TESTS	MEAN METABOLISM RATE	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Totonicapan	134	M	Basal	+8.20±0.958	5.24±0.674	63.92± 8.24
Totonicapan	20	M	3 hours after chili feeding	+8.46±1.34 [2]	6.01±0.949	71.03±11.22
Totonicapan	12	M	3 hours after tortilla feeding	+1.30±2.10	7.29±1.48	58.90±12.02
Samayac laborers	30	M	Basal	+5.20±1.14	6.26±0.808	120 ±15.5
White	2	F	Basal	−6		
White	6	M	Basal	−11.5±1.21	2.98±0.861	30.84± 8.90

[2] Increase over subjects' own basal rate.

individuals of similar sex, age, weight and height. The mean value of +8.20 ± 0.958% secured on the soldiers, we take to be a close approach to the true basal metabolic rate for this group. The tests were run at from 11.5 to 16°C. open

[1] The gauge of this apparatus delivers a constant volume of oxygen under varying degrees of temperature and pressure. In repeated comparative tests at varying pressures with a water spirometer we have found the variations in metabolism readings to run less than 3%. We have measured the accuracy of the machine at barometric pressures as low as 54.5 mm. of Hg. In repeated tests we find that the gauge measures within 1% the volume of the gas as measured under standard conditions with the water spirometer.

air temperatures. Since the subjects were well blanketed and appeared comfortable at all times, we believe that the outside temperature did not increase their basal metabolic rate. Apparently this temperature is within their habitat temperature range. By habitat temperature we include that temperature range to which the individual or the animal is normally subjected and apparently adapted. The barometric pressure which averaged 506 mm. of Hg. for six consecutive daily readings apparently does not affect the basal metabolic rate to any extent for the tests on the laborers at 800 feet altitude averaged $+5.20 \pm 1.14\%$. Five tests under basal conditions upon one white female and one white male subject at 8100 feet altitude gave an average value of -7.5%. Three tests upon another white male at 800 feet gave a value of -10%.

The twenty tests made 3 hours after chili feeding showed an average percentage increase over the subject's own basal rates of $+8.46\%$ while tortilla feeding increased the rate by $+1.30\%$.[2]

The average values for both the Maya soldiers and laborers are in keeping with the values of Benedict ('29) of $+5.2\%$ on thirty-two male Mayas at Chichin Itza Yucatan, secured by Williams and Benedict ('29) and with those of Shattuck and Benedict ('30) viz., $+5.8\%$ on thirty Indian subjects. Steggerda ('32) verified these earlier findings and secured an average of $+8\%$ above normal white metabolism standards.

It appears from the findings of MacLeod, Earle, Necheles, Heinbecker, Okada, Van Berkhout, to mention only a few workers in addition to those already cited, that definite racial differences in basal metabolic rates exist. A striking fact, however, is that the Indian of Yucatan, Guatemala and

[2] Recently we have tested the effect of chili on a number of guinea pigs and human subjects. Our maximum dosage was 1½ gm. of dry chili for the human subject and up to ½ gm. for the guinea pigs. These tests on the human subjects have given negative results to date. In the case of the guinea pigs, our first tests were negative but continued dosage over about 3 weeks increased the basal metabolic rate from 12 to 14% in some cases. It is possible that continued use of chili might have a stimulatory effect on the thyroid glands since there is evidence of hyperplastic change in some of our treated pigs.

the Navajo [3] of Arizona and the Eskimo, all run counter to the findings made on the Mongolians of China, Japan and Java.

BLOOD PRESSURES AND PULSE RATES

The blood pressure on the soldiers averaged 111 systolic and 77 diastolic, on the laborers it averaged 104 and 73. The average for the white subjects with an average age of 31.6 years was 126 systolic and 74 diastolic. The pulse rates on the soldiers ran 63 before and 61 after the tests while those of the laborers ran 62 before and 60 after. The white female had a pulse rate of 72 while the white males averaged 68.

GOITER

We have observed a high incidence of diffuse endemic goiter throughout the highland regions. All the Indian subjects with the exception of nos. 22 and 26 were examined by Doctor Crile for goiter, only in no. 31 was no evidence of goiter found.

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[3] In a personal communication from Dr. C. G. Salsbury of the Sage Memorial Hospital, Ganado, Arizona, he states, "We have recently compiled figures on 1136 basal metabolisms (Navajos) and these show an average of +2.4%; 1277 blood pressures show a systolic of 111.9 and a diastolic of 71.6."

THE RELATION OF VITAMIN C DEFICIENCY TO NUTRITIONAL ANEMIA [1]

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ONE FIGURE

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The occurrence of anemia in scurvy has been recognized for a long time. However, the degree of anemia very rarely parallels the other symptoms which are regarded as characteristic for scurvy (Hess and Fish, '14; Hess, '20; Aron, '22; Mettier, Minot and Townsend, '30; Rohmer and Bindschedler, '32; and Parsons and Smallwood, '35).

The frequent and simultaneous occurrence of anemia and scurvy has an important bearing on the cause of nutritional anemia. A study by Aron ('27) of the cases of nutritional anemia treated between 1908 and 1926 in the University Children's Hospital at Breslau revealed that during the period of frightful shortage of food material, 1921 to 1923, there was an extraordinary increase in the number of cases admitted for severe anemia coincident with a hitherto unknown number of cases of scurvy observed during the same period. Fifteen cases of anemia were carefully examined for scurvy according to clinical symptoms and x-ray findings. Such symptoms were found in seven instances (Aron, '22). It is remarkable that Rohmer and Bindschedler ('32), recording the results of blood examinations in fifteen cases of infantile scurvy, found anemia in 'only seven instances.'

Before ascorbic acid was discovered only so-called 'antiscorbutic foodstuffs,' like orange, lemon, or other fruit juices,

[1] This investigation was conducted under a grant from the Emergency Committee in Aid to Displaced Foreign Medical Scientists.

green vegetables or germinated oats, all of them containing a number of other constituents, could be used in clinical or experimental investigations. Therefore, it was not possible to decide which constituent of the so-called 'antiscorbutics' was the active factor causing anemia by deficiency and acting as a hemopoietic when added to the diet.

After vitamin C became available in chemically pure form as ascorbic acid, some investigations were made concerning its influence upon the blood, especially upon the red blood cells and reticulocytes. However, the results reported thus far are contradictory in several respects and the fundamental problems are not satisfactorily solved (Jonas, '23; Meyer and McCormick, '28; Mettier, Minot and Townsend, '30; Mettier and Chew, '32; Rohmer and Bindschedler, '32; Dunlop and Scarborough, '35; Parsons and Smallwood, '35; Euler and Malmberg, '36, '37, '38; Aszodi, '37; Kenney and Rapoport, '38; Mettier, '38, and Ungley, '38).

Since the fundamental problem of the relationship of ascorbic acid to blood formation and to anemia cannot be solved by clinical studies alone, a systematic experimental investigation is necessary. There are very detailed procedures and rigid rules for the study of the different scorbutic symptoms in experimental scurvy of guinea pigs. These include a study of the body weight, the post mortem findings on the various organs, the bones and even the histological structure of the teeth. However, until now no attempt has been made to include a study of the hemoglobin and the blood cells in the 'biological assay' for vitamin C. It is obvious that for the purpose of 'biological assay' such blood examination if applicable would offer certain advantages over methods based only on post mortem findings or histological examination.

EXPERIMENTAL PROCEDURE

a. First series. In the first series of experiments the method for biological standardization of vitamin C was strictly followed (Coward, '36, '38; King, '38).

The scorbutogenic diet (diet sc.) used throughout all experiments was prepared every 10 to 20 days by mixing the following components:

Heated skimmed milk powder[1]	300
Ground whole oats	590
Melted butterfat, strained	90
Cod liver oil	10
NaCl	10

In addition to diet sc., 5 gm. dry oats were given daily in the first and second series of experiments.

Four male guinea pigs (nos. 3, 4, 11 and 12) somewhat over 200 gm. in body weight were kept on a diet of oats and carrots for a preliminary period. Hemoglobin and red blood cells were checked at least twice. When the animals had reached weights of 265, 275, 280 and 300 gm. respectively they were transfered to the scorbutogenic diet.

The scorbutogenic diet produced the well-known signs of scurvy. A distinct reduction of hemoglobin with a slight reduction of the R.B.C., however, developed in only one of these animals. The other three animals showed no distinct anemia in spite of the fact that they had lost nearly 25% of their body weight. None of those four animals could be saved even by the administration of ascorbic acid subcutaneously or orally in doses of 25 mg. daily. These experiments agree fully with those performed by Dr. M. I. Pierce[2] in this laboratory.

From earlier experiments (Aron, '22, and Jonas, '23) we had the impression that young guinea pigs or guinea pigs of lower body weight do not become anemic as readily as do older ones of higher body weight. A critical analysis of the experiments published by Meyer and McCormick ('28) seemed

[1] The skimmed milk powder was heated in a very thin layer in flat pans for at least 4 hours. During the heating process the powder was stirred and turned over several times. The heating was continued until the milk powder showed a pronounced brown color. The purpose of this was to destroy completely vitamin C and such other heat labile factors as might be present, as e.g. the 'grass juice factor' (Koehler, Elvehjem and Hart, '38).

[2] Unpublished results.

to confirm this belief. For this reason in the next series of experiments the guinea pigs were not put on the deficiency diet before they had grown to about 500 gm. and were at least 4 months of age.

b. Second series. This experiment included six guinea pigs, all of them male (nos. 1, 2, 5, 7, 6, 10). In a preliminary period carrots and oats with milk were given. Then the animals were transfered to the scorbutogenic diet which for a limited period was supplemented either by addition of 5 gm. fresh germinated oats [4] daily, or by 12.5 mg. ascorbic acid [5] dissolved in 2 to 3 cc. of water and fed orally.

On the one hundred and first day the administration of vitamin C was discontinued and dry oats was substituted for germinated. Following this change a distinct anemia was produced in all six animals as will be seen from table 1. All these animals were in the ultimate stage of vitamin C deficiency. When we attempted to cure the anemia even amounts up to 100 mg. ascorbic acid, given either by mouth or by subcutaneous injection, did not produce a remission of the anemia. Four animals, nos. 1, 2, 5 and 7 succumbed within 3 to 6 days. However, pigs nos. 6 and 10 which on the fourth day were placed on a diet of germinated oats and concentrated milk could be saved.

In spite of the fact that the quantity of ascorbic acid contained in the germinated oats was less than 5 mg. there was a marked improvement immediately after this change of diet was introduced. After the animals had been on this supplement of 5 gm. germinated oats for a period of 10 days, the germinated oats were replaced by ascorbic acid. At first 25 mg., later 2 mg. daily were fed orally to each animal.

Before the guinea pigs had again reached their original weight the hemoglobin content of the blood was within normal limits.

[4] Five grams germinated oats, according to our chemical determination, have an average content of about 2 mg. ascorbic acid.

[5] We wish to express our appreciation to Merck and Co., Rahway, N. J., for furnishing the ascorbic acid (Cebione) used in this investigation.

c. A third series of experiments was initiated with the intention of starting the supplement with the anemic animals before they reached the ultimate (fatal) stage. Six male guinea pigs all of whom had been watched closely for more than 100 days were fed our scorbutogenic diet (diet sc.) with an addition of either ascorbic acid (Cebione) or a juice pressed from germinated oats for 53 to 68 days respectively.

TABLE 1

NUMBER OF ANIMAL	SUPPLEMENT TO DIET SC.	AVERAGE BEFORE WITHDRAWAL OF ANTISCORBUTIC		LOWEST VALUE AFTER WITHDRAWAL OF ANTISCORBUTIC
1	68th to 100th day: 5 gm. dry oats +12.5 mg. ascorbic acid	Hgb. gms.	14.9	8.2
		Ery. mill.	4.5	2.2
2	68th to 100th day: 5 gm. dry oats +12.5 mg. ascorbic acid	Hgb. gms.	14.8	12.4
		Ery. mill.	4.6	3.7
6	82nd to 100th day: 5 gm. dry oats +12.5 mg. ascorbic acid	Hgb gms.	14.2	11.1
10	82nd to 100th day: 5 gm. dry oats +12.5 mg. ascorbic acid	Hgb. gms.	14.5	10.6
5	68th to 100th day: 5 gm. germinated oats	Hgb. gms.	15.4	11.6
		Ery. mill.	4.9	4.3
7	68th to 100th day: 5 gm. germinated oats	Hgb. gms.	15.9	11.8
		Ery. mill.	4.8	4.3
Average	Hemoglobin, grams		14.9	11.0 = 26% reduction
	Erythrocytes, mill.		4.7	3.7 = 21% reduction

The life duration of a red blood cell hardly amounts to more than 50 days. Consequently we are fully justified in stating that the red blood cells of these guinea pigs and therefore the hemoglobin within these cells was built up entirely while these animals were on diet sc. with the respective supplement.

As the anemia produced by the withdrawal of vitamin C is always of a distinctly hypochromic type, the decrease in

hemoglobin is comparatively greater than that in the number of red blood cells (see also table 1). For this reason it seemed highly important to concentrate our studies upon the hemoglobin changes. In order to make the hemoglobin determinations as accurate as possible they were performed in duplicate with two different types of hemoglobinometers, the one checking against the other (Newcomer and Hellige or Sahli Leitz). Blood was drawn at intervals from 2 to 5 days.

In guinea pigs nos. 6 and 10 the withdrawal of the ascorbic acid supplement produced anemia for a second time; the loss of hemoglobin amounting to 24% and 30% respectively.[6] No attempt was made to cure these animals which were made anemic for a second time.

With the other four animals of this group, however, we gave the ascorbic acid anew in sufficient time to cure the animals. Once or twice daily 50 mg. of ascorbic acid were given either subcutaneously[7] or orally. With these large doses all four animals were saved.

Following this administration of ascorbic acid, a surprisingly fast regeneration of hemoglobin was observed. When the weight curves began to rise, the hemoglobin levels had regained their normal values. The most important figures are compiled in figure 1.

Throughout all experiments it was noticed that the more the anemia develops the more difficult it becomes to obtain sufficient amounts of blood for hemoglobin determinations by pricking the ears of the guinea pigs. However, within a few days after ascorbic acid is given anew, there is again ample bleeding from the same ears from which a short time before, with exactly the same technic, blood could be obtained only with great difficulty. This may be due to a reduction of the total

[6] These animals in addition to the scorbutogenic diet were given an extra supplement of iron from the time the first decline in hemoglobin was noticed. Iron pyrophosphate in doses of 0.2 to 0.5 gm. daily were given in the drinking water. This mode of iron administration was adopted since the animals while declining in weight as well as hemoglobin still drank some water, while the food was hardly touched.

[7] 'Cenolate' kindly furnished by Abbott Laboratories, North Chicago, Ill.

blood volume. It may also be due to desiccation. Such desiccation would explain why some investigators have reported an increase of red blood cells and of hemoglobin in clinical as well as in experimental scurvy (Hess and Fish, '14; Hess, '20 and Aszodi, '37). In our experiments there was also a slight rise noted in some instances after the lowest values for hemoglobin were obtained.

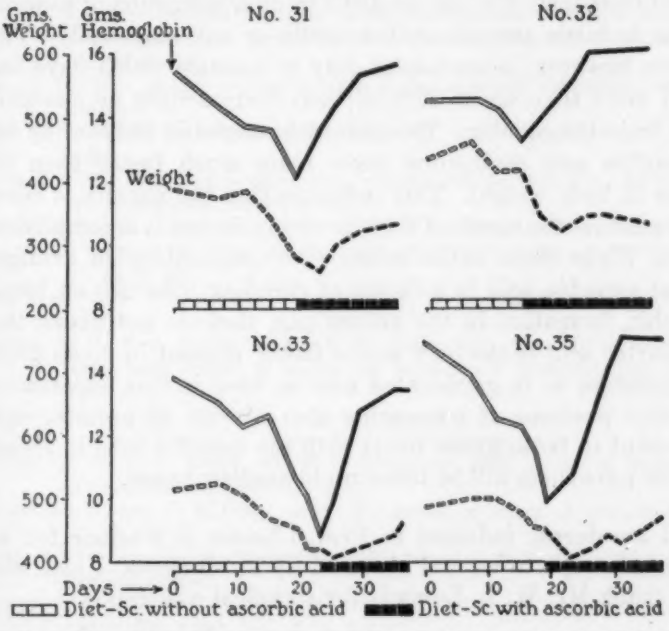

Fig. 1 For each graph: Upper curve: Hemoglobin. Lower curve: Weight.

SUMMARY

1. When guinea pigs are fed a scorbutogenic diet with an addition of ascorbic acid for 50 days or more, a period longer than the life cycle of the erythrocyte, normal blood formation takes place.

2. When the ascorbic acid supplement is withdrawn, guinea pigs 4 months of age having a body weight of 450 gm. or more

show a distinct reduction in the hemoglobin content of the blood within 20 days. A supplement of iron does not prevent this decline in hemoglobin. Younger animals, of 200 to 300 gm. body weight, may also become anemic. However, they very rarely do so because they usually succumb before a distinct anemia develops.

3. Guinea pigs made anemic by the withdrawal of ascorbic acid from their diet can be cured by administration of ascorbic acid in large amounts either orally or subcutaneously. This cure, however, is successful only in animals which have lost not more than about 25% of their body weight or one-third of their hemoglobin. The rise in hemoglobin induced by the ascorbic acid medication takes place much faster than the rise in body weight. This indicates that the anemia is cured long before the repair of the other body tissues is accomplished.

4. While these experiments give unquestionable evidence that ascorbic acid is a factor of deciding influence on hemoglobin formation in the guinea pig, they do not prove that ascorbic acid is the only active factor present in fresh green vegetables or in germinated oats as used in this experiment.

The problem of comparing the activity of ascorbic acid present in fresh green foods with the ascorbic acid in chemically pure form will be taken up in another paper.

I am deeply indebted to Prof. Chester J. Farmer for his advise, cooperation and his courtesy at all times. I also wish to thank Mr. W. L. Lummis for technical assistance.

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UTILIZATION OF ENERGY OF WHEAT PRODUCTS BY CHICKENS[1]

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The energy values of feeds and foods were formerly compared on the basis of the chemical analyses for protein, ether extract, crude fiber and nitrogen-free extract. Next in point of time they were compared on the basis of digestible nutrients, with the assumption that the digestible nutrients of the same class have the same value to the animal, regardless of origin. This procedure is still being used (Morrison, '36), though experimental evidence with cattle is conclusive that such equality often does not exist and that the digestible constituents of some feeds, such as roughages, sometimes have a value of half or less of the digestible constituents of others, such as concentrates. The calories of human foods are now usually calculated (Morey, '36; Rose, '38; Sherman, '37) by multiplying the grams of protein by 4, the fat by 9 and the carbohydrates (which sometimes includes the crude fiber) by 4. The calories for human food obtained by such calculations, sometimes called the physiological fuel value (Sherman, '36), are presumably the metabolizable energy, which is the total energy less the energy lost in the solid and liquid excrements. This method of calculating the energy values of human foods was devised for a mixed diet (Morey, '36) and when applied to single foods incorrectly assumes that all foods are equally digestible and also assumes that the energy values of the metabolizable energy of different feeds are equal. As will be shown in this paper, this assumption is also incorrect.

[1] Read at the Baltimore meeting of the American Chemical Society, April 6, 1939.

The energy values of feeds, as measured by the energy of the fat and flesh which can be produced on fattening cattle when the feeds are added to a diet already sufficient for maintenance, have been extensively studied and for a long time have been used for comparing energy values of cattle feeds, formulating feeding standards, etc. (Armsby, '17; Forbes and Kriss, '25; Fraps, '11, '25, '28; Kellner, '05). Such energy values, expressed in therms per 100 pounds of feed, have been called the productive energy by Fraps and the net energy by Armsby and by Forbes. Only a few estimates of the productive energy of chicken feeds have been made. Mitchell and Haines ('37) estimated the net energy of corn for chickens to be 2.83 calories per gram, Southgate ('29) calculated that a mixture of 13 parts oats and 3 parts dried whole milk has a net energy of 1.8 calories per gram, and Fraps and Carlyle ('39) found the net energy of a ration for chickens to be 1.79 calories per gram. The work here reported is a comparison of the productive energy values of patent flour, low-grade flour, wheat bran and wheat brown shorts, with cornmeal as a standard as measured by the energy of the protein and fat gained by young growing chickens.

METHODS

Four groups of six chickens each were used in the tests. One group was fed a ration containing 50% of cornmeal, while the other three were fed rations containing 50% of the feed to be tested. The remainder of the ration was the same in all four groups and all the other conditions were made as uniform as possible, so that the only variable was the feed being tested. The basal 50% of the ration consisted of 20% wheat brown shorts, 12% casein, 6% cornmeal, 6% alfalfa leaf meal, 2% yeast, 1% calcium carbonate, 1% salt, 1% tricalcium phosphate and 1% of fortified fish oil. Sixty or more baby chicks were fed in a preliminary period of 1 week on the cornmeal ration, and then four groups of six each were selected so as to have the same average weight. Four of the remaining chicks, as nearly similar to the others as possible, were ana-

lyzed at the beginning of the experiment, and the other remaining chicks were used for digestion experiments on the rations being studied by methods previously used (Fraps, '28). The experimental chicks were placed in individual compartments in battery brooders, fed individually for 3 weeks, and weighed at the end of each week. They were then killed, the intestinal contents removed, and the entire chick ground up together with about 1% boric acid and 3% filter paper pulp. Protein (N × 6.25) and fat were determined at

TABLE 1

Percentage composition of feeds

	EXPERIMENT NUMBER	PROTEIN	ETHER EXTRACT	CRUDE FIBER	NITROGEN FREE EXTRACT	WATER	ASH
Cornmeal	6–6	11.05	3.00	1.39	73.15	9.93	1.48
Cornmeal	6–7	9.86	4.11	1.36	72.78	10.84	1.05
Cornmeal	6–8	11.88	4.96	1.58	69.63	10.27	1.68
Patent flour	6–6	12.57	0.91	0.25	73.25	12.55	0.47
Patent flour	6–7	12.83	0.84	0.24	73.43	12.17	0.49
Patent flour	6–8	13.96	1.01	0.40	70.88	13.22	0.53
Low-grade flour	6–6	13.20	2.24	0.43	71.67	11.52	0.94
Low-grade flour	6–7	15.07	1.94	0.50	70.13	11.46	0.85
Low-grade flour	6–8	18.78	2.08	0.50	64.89	12.74	1.01
Wheat bran	6–6	17.87	4.18	10.13	52.47	8.93	6.42
Wheat bran	6–7	19.24	4.05	9.41	50.64	10.33	6.33
Wheat brown shorts	6–8	19.03	4.24	6.43	54.83	10.51	4.96
Wheat brown shorts	6–6	19.33	4.77	6.69	54.95	9.53	4.73
Wheat brown shorts	6–8, 6–7	19.50	4.62	6.47	54.94	9.57	4.90

once, not less than three determinations being made on each chick. After correcting for additions of boric acid and paper pulp, the energy content was calculated by use of the factors 5.66 for protein and 9.35 for fat. This method was found by Fraps and Carlyle ('39), to give results agreeing with the heats of combustion determined in a bomb calorimeter.

RESULTS OF WORK

Three experiments are here reported. The composition of the feeds used is given in table 1. Analyses of the rations

also were made and had practically the same composition as those calculated from the analyses in table 1 and the percentages of the ingredients.

The weights and average composition of the chicks and other data for the three experiments are given in table 2. At

TABLE 2

Composition and weights of chickens

	LIVE WEIGHT AT BEGINNING	LIVE WEIGHT AT END	EMPTY WEIGHT AT END	PROTEIN	FAT	CALORIES PER 100 GM. EMPTY WEIGHT
	gm.	gm.	gm.	%	%	
Beginning of experiment 6–6	65.5	65.5	56.3	16.74	7.42	164.1
Calories per 100 gm. live weight 141.2						
Cornmeal ration	65.9	191.7	181.4	20.09	8.81	196.1
Patent flour ration	65.6	187.6	169.7	20.14	7.75	186.4
Low-grade flour ration	65.9	181.6	173.8	20.34	6.15	172.6
Wheat bran ration	65.8	177.2	167.9	20.84	2.96	145.6
Beginning of experiment 6–7	51.5	51.5	49.5	16.93	5.23	144.7
Calories per 100 gm. live weight 139.2						
Cornmeal ration	53.7	167.7	163.8	20.32	8.37	192.4
Patent flour ration	52.9	161.1	156.9	20.93	6.13	175.8
Low-grade flour ration	52.9	173.4	169.6	21.25	6.23	178.5
Wheat bran ration	53.1	159.0	152.2	21.70	2.83	149.2
Beginning of experiment 6–8	59.9	59.9	58.2	18.17	6.79	166.3
Calories per 100 gm. live weight 161.4						
Cornmeal ration	60.5	209.7	203.2	20.34	8.04	190.3
Patent flour ration	59.6	194.7	189.7	20.93	7.04	184.3
Low-grade flour ration	59.9	201.0	194.7	20.79	5.04	164.8
Wheat brown shorts ration	60.4	199.9	193.9	21.03	3.70	153.7

the end of the experiment the chicks fed the wheat bran ration averaged only about 3% fat and those fed on the wheat brown shorts ration averaged only 3.7% fat as compared with more than 8% fat in the chickens fed on the cornmeal ration. There are great differences in fat and energy content for the different rations. The live weights at the end of the experiment

averaged 191.7 gm. for the cornmeal ration, compared with 177.2 for the wheat bran ration in experiment 6, and 167.7 gm. compared with 159.0 in experiment 7. The energy value of the ration may thus affect the fatness of the chickens more than it affects the live weight.

The results of the chemical analyses, the digestion experiments and the estimation of metabolizable energy are summarized in table 3, both for the rations and for the feeds.

TABLE 3

Effective organic nutrients, effective digestible nutrients and metabolizable energy per 100 gm. of rations and of feeds

		RATIONS			FEEDS		
		Effective organic constituents	Effective digestible nutrients	Metabolizable energy	Effective organic constituents	Effective digestible nutrients	Metabolizable energy
		gm.	*gm.*	*cal.*	*gm.*	*gm.*	*cal.*
Basal part of ration		82.0	59.0	274.0	..	..	..
Cornmeal	Exp. 6	86.4	70.5	319.3	91.0	82.0	364.6
Patent flour	Exp. 6	85.8	68.5	307.0	87.9	78.0	340.0
Low-grade flour	Exp. 6	86.5	66.6	299.9	89.9	74.2	325.8
Wheat bran		83.2	47.9	213.1	79.8	36.8	152.2
Cornmeal	Exp. 7	86.9	68.0	322.3	91.9	77.0	370.0
Patent flour	Exp. 7	86.5	69.4	311.7	88.2	79.8	349.4
Low-grade flour	Exp. 7	85.8	68.3	313.0	89.6	77.6	352.0
Wheat bran	Exp. 7	80.8	45.9	208.3	79.0	32.8	142.6
Cornmeal	Exp. 8	88.6	71.2	320.4	92.7	83.4	366.8
Patent flour	Exp. 8	86.8	69.9	319.1	87.1	80.8	364.2
Low-grade flour	Exp. 8	85.6	65.2	293.2	88.4	71.4	312.4
Wheat brown shorts	Exp. 8	83.6	53.5	241.8	83.4	48.0	209.6

The effective organic constituents are the sum of the protein, the ether extract multiplied by 2.25, and the nitrogen-free extract, as found in the analyses made of the rations and of the feeds. The crude fiber is considered to have no energy value for chickens. The effective digestible nutrients are the sum of the digestible protein, the digestible ether extract multiplied by 2.25 and the digestible nitrogen-free extract. For the rations they were calculated directly from the average data of two digestion experiments. For 100 parts of the feeds

they were obtained by deducting the effective digestible nutrients of one-half of the basal part of the ration from those of the total ration and multiplying by 2. For example, for cornmeal in experiment 6, 59.0 divided by 2 and the result subtracted from 70.5 and multiplied by 2 gives 82.0, the effective digestible nutrients in 100 parts of the cornmeal. The metabolizable energy is the gross energy of the feed minus the energy of the excrement, since with the chickens the excrement contains the urinary excretion as well as the undigested material. The metabolizable energy of the rations was determined in connection with the digestion experiments and that of 100 parts of the feeds by subtracting one-half the

TABLE 4

Maintenance requirements calculated from the cornmeal ration

EXPERIMENT NUMBER	AVERAGE WEIGHT OF CHICKS BY PERIODS	INITIAL ENERGY CONTENT OF CHICKS	FINAL ENERGY CONTENT OF CHICKS	GAIN OF ENERGY OF CHICKS	PROD. ENERGY OF RATION	TOTAL RATION EATEN	PROD. ENERGY OF FEED EATEN	FOR MAINTENANCE	
								Total prod. energy	Prod. energy per period and 100 gm. chicken
	gm.	*cal.*	*cal.*	*cal.*	*cal. per gram*	*gm.*	*cal.*	*cal.*	*cal.*
6	118.0	93.1	357.5	264.4	1.96	275.2	539.4	275.0	234.5
7	107.3	74.7	315.5	240.8	1.89	249.0	470.6	229.8	215.2
8	131.9	97.6	386.8	289.2	1.98	288.6	571.4	282.2	214.2

metabolizable energy of the basal part of the ration and multiplying by 2 as was done with the effective digestible nutrients. The determinations of heats of combustion for this work were made in an Emerson bomb calorimeter by Dr. J. F. Fudge.

Data for the calculations for the maintenance requirements and the value of the rations for fattening are given in tables 4 and 5. The maintenance requirements are calculated with use of the average weights by periods. The average weight by periods is the average of the average weight for each of the 3 weeks, secured by the formula $\frac{a+b}{2} + \frac{b+c}{2} + \frac{c+d}{2}$, divided by 3, in which a is the live weight at the beginning, and b, c,

and d, the weights at the end of the first, second and third week respectively. The use of the average weight by periods has been shown by Fraps and Carlyle ('31) to give more consistent results that the use of the average of the first and last weights. The use of the weight as a basis for calculating the maintenance requirements was also shown by Fraps

TABLE 5

Calculation of productive energy of rations

	EXPERIMENT NUMBER	AVERAGE WEIGHT OF CHICKS BY PERIODS	INITIAL ENERGY CONTENT OF CHICKS	FINAL ENERGY CONTENT OF CHICKS	GAIN OF ENERGY OF CHICKS	RATION EATEN	USED FOR MAINTENANCE	FOR GAIN AND MAINTENANCE	PRODUCTIVE ENERGY OF RATION
		gm.	*cal.*	*cal.*	*cal.*	*gm.*	*cal.*	*cal.*	*Calories per 100 gm.*
Patent flour ration	6	111.6	92.6	316.2	223.6	271.9	262.2	485.8	179.2
Low-grade flour ration	6	115.4	93.1	302.1	209.1	277.2	271.1	480.2	173.7
Wheat bran ration	6	114.6	93.0	244.8	151.8	356.5	268.8	420.6	117.8
Patent flour ration	7	103.8	73.6	276.6	203.0	249.0	223.3	426.2	171.8
Low-grade flour ration	7	111.6	73.6	303.4	229.8	260.6	239.9	469.6	180.0
Wheat bran ration	7	106.5	74.0	228.4	154.4	355.2	228.9	383.3	107.2
Patent flour ration	8	128.4	96.1	351.8	255.0	299.8	274.8	529.8	176.4
Low-grade flour ration	8	127.7	96.7	321.6	224.9	289.2	273.3	498.2	172.3
Wheat brown shorts ration	8	124.1	97.5	297.9	200.4	376.1	265.7	466.1	125.0

and Carlyle ('39) to give results better in accord with previous work of others than the use of the surface area.

For the purpose of comparing the energy values of the other feeds with that of cornmeal, 1 gm. of the effective digestible nutrients of the cornmeal ration and of the cornmeal consumed over maintenance was assumed to produce 2.78 calories of gain in the chicken. This is the average value secured by Fraps and Carlyle ('39) for a mixed ration con-

taining 51% cornmeal, 19% wheat brown shorts, 10% dried buttermilk, 6% cottonseed meal, 5% alfalfa leaf meal, 4% tankage, 2% bone meal, 2% oyster shell and 1% salt. As the equality of the digestible nutrients of the mixture is assumed, and as the work here reported shows that the value of the digestible nutrients of wheat brown shorts is much below that of cornmeal, the 2.78 calories per gram of digestible nutrients is too low for the cornmeal ration. However, correction of this value must await more data as to the value of the energy contained in the various ingredients of the ration used by Fraps and Carlyle ('39). Since the object of this work is to secure comparative energy values of the feeds tested, rather than absolute values, the use of too low a value for productive energy will not introduce any large error. The productive energy of the cornmeal ration given in table 4 was secured by multiplying the effective digestible nutrients of table 3 by 2.78.

Since cornmeal is used as the standard, the calories of productive energy used for maintenance under the conditions of each experiment are calculated from the cornmeal ration, as given in table 4. The initial energy content of the chicks is calculated from the initial live weight and the initial energy per gram, as found by analysis (table 2) and the final energy content from the final empty weight and final energy content of the chicks. The productive energy of the ration eaten is the grams eaten multiplied by the productive energy (1.96 in case of experiment 6). The productive energy used for maintenance is the productive energy of the ration eaten less the gain of energy in calories, of the chicks, since by definition, the productive energy is measured by the gain in energy. The maintenance requirements are calculated to calories of productive energy required to maintain 100 gm. of chicken for the period of each experiment.

The maintenance requirements calculated in table 4 are used in table 5 to calculate the productive energy of the rations. The method of procedure should be obvious from the headings of the table. The sum of the calories of productive

energy used for gain and for maintenance divided by the quantity of feed eaten and multiplied by 100 gives the calories of productive energy of 100 gm. of the ration, and these are given in the last column of table 5.

TABLE 6

Comparative productive energy of feeds in calories

EXPERIMENT NUMBER	CORNMEAL	PATENT FLOUR	LOW-GRADE FLOUR	WHEAT BRAN	WHEAT BROWN SHORTS
Productive energy per 100 gm. of feed					
6	228	194	183	71	..
7	214	179	196	50	..
8	232	189	181	..	86
Average	225	188	187	61	86
Productive energy per 100 gm. of effective organic constituents					
6	251	221	204	89	..
7	233	203	219	63	..
8	260	217	205	..	103
Average	248	214	209	76	103
Productive energy per 100 gm. of effective digestible nutrients					
6	278	249	247	193	..
7	278	224	253	152	..
8	278	234	254	..	179
Average	278	236	251	173	179
Productive energy per 100 calories of metabolizable energy					
6	63	57	56	47	..
7	58	51	56	35	..
8	63	52	58	..	41
Average	61	53	57	41	41

The averages given in tables 5 and 6 are calculated from the data for individual chickens. The results would be slightly different had average data been used in the calculating of the maintenance requirements or of the productive energy.

The difference between the assumed productive energy value of 100 gm. of the corn meal ration (196 cal.) and of

the patent flour ration (179) gives the effect (—17) of substitution of 50 gm. of patent flour for 50 gm. of cornmeal. Twice this difference (—34) added to the productive energy of the cornmeal (228) gives the productive energy of 100 gm. of the patent flour (194 cal.). The productive energy values of the other feeds were calculated in a similar way.

TABLE 7

Productive energy of feeds compared with cornmeal as 100

EXPERIMENT NUMBER	CORNMEAL	PATENT FLOUR	LOW-GRADE FLOUR	WHEAT BRAN	WHEAT BROWN SHORTS
Productive energy per 100 gm. of feed					
6	100	85	80	31	..
7	100	84	92	23	..
8	100	81	78	..	37
Average		83	83	27	37
Productive energy per 100 gm. of effective organic constituents					
6	100	88	81	35	..
7	100	87	94	27	..
8	100	83	79	..	40
Average		86	85	31	40
Productive energy per 100 gm. of effective digestible nutrients					
6	100	90	89	69	..
7	100	81	91	55	..
8	100	84	91	..	64
Average		85	90	62	64
Productive energy per 100 calories of metabolizable energy					
6	100	90	89	75	..
7	100	88	97	60	..
8	100	83	92	..	65
Average		87	93	68	65

The productive energy values of the feeds are given in the top section of table 6. By means of these data and the data in table 3 calculations have been made for the productive energy in calories per 100 gm. of effective organic constituents, of effective digestible nutrients and of 100 calories of metabolizable energy.

DISCUSSION OF RESULTS

With the average productive energy of cornmeal at 225 calories per 100 gm., the average productive energy of the patent flour as given in table 6 is 188 calories, that of the low-grade flour is 187, that of the wheat brown shorts is 86 and that of the wheat bran is 61. The productive energy of wheat bran is a little more than one-fourth of that of cornmeal. The average productive energy per 100 gm. effective digestible nutrients are the assumed value of 278 for the cornmeal, 236 for the patent flour, 173 for the wheat bran and 179 for the wheat brown shorts. That is, the energy value per gram of digestible constituents differs in different feeds and the quantity of digestible constituents is not a correct measure of the value of a chicken feed for growth. It is also seen in table 6 that 61% of the calories of metabolizable energy are utilized from cornmeal but only 41% from wheat bran or wheat brown shorts. The metabolizable energy is not a correct measure of the value of a feed for growth and production.

Compared with that of cornmeal at 278 calories the productive energy per 100 gm. of digestible nutrients of the patent flour averages 236 and that of the low-grade flour 251. It is impossible at the present time to say why the digestible nutrients of flour have lower productive energy than cornmeal. The chemical compounds (chiefly starch and protein) do not appear to be sufficiently dissimilar in quantity or quality to cause these differences. The possibility of a vitamin deficiency in the rations is being investigated.

The productive energy value per unit of the digestible nutrients of the wheat brown shorts and of wheat bran are 64% and 62% of those of cornmeal. These lower values perhaps may be partly accounted for on the assumption that the products of the digestion of the pentosans and hemicelluloses present in wheat bran and wheat brown shorts are not as completely utilized by the animal body as the sugars formed from the digestion of the starches of cornmeal and wheat flour. This explanation, of course, requires further study.

The relative productive energy of the metabolizable energy of low-grade flour is 57, of patent flour is 53, of wheat brown shorts is 41 and of wheat bran is 41, compared with that of cornmeal at 61. The metabolizable energy is a little better measure of productive energy than the digestible nutrients but not sufficiently accurate for use. When measured by gains of protein and fat on growing chickens, neither the total nutrients, the digestible nutrients, nor the metabolizable energy are correct measures for the relative values of cornmeal, wheat flour, wheat brown shorts and wheat bran. Since the difference between the productive energy and the metabolizable energy must be dissipated as heat, wheat bran and wheat brown shorts should produce a greater excess of heat to dissipate than cornmeal or wheat flour, and this fact may be especially important in warm climates.

The human digestive organs are different from those of chickens, and the quantity of material digested by a human from a given food may be different from that digested by a chicken from the same food. However, once the food has been digested, there may be little relative difference in utilization of the energy of the digested materials. The results here presented at least indicate that wheat bran may have a low value in human food and that the removal of the bran (about 18% of the whole wheat grain) and of wheat brown shorts (about 12%) in the process of flour milling may be an advantage. They indicate that graham flour or whole wheat flour may have about 90% of the energy value of white flour. They indicate that cornmeal, wheat flour, graham flour and whole wheat flour may differ in productive energy for human food and not be equal in energy value as is at present assumed to be the case (Morey, '36; Sherman, '37; Rose, '38) and that the relative energy values now used need to be studied and revised.

SUMMARY

The value of several feeds for maintenance and for production of gain when fed to young growing chicks was compared with the value of cornmeal. The feeds to be compared

were fed as half of a ration otherwise constant. The energy content was calculated from analyses made for protein and fat on representative chicks at the beginning and the chicks on experiment after 3 weeks of the feeding tests. The maintenance requirements were calculated from the data secured with the cornmeal ration. The calories of energy gained were calculated from the data of the other tests made at the same time. With the cornmeal ration as a standard, three tests were made with patent flour and low-grade flour, two with wheat bran and one with cornmeal. With the productive energy of cornmeal at 225 calories per 100 gm., the relative productive energy values of the other feeds averaged: patent flour 188, low grade flour 187, wheat brown shorts 86 and wheat bran 61. The relative productive energy values of effective digestible nutrients averaged, with cornmeal at 278, in patent flour 236, in low-grade flour 251, in wheat brown shorts 179 and in wheat bran 173. The relative productive energy values of the metabolizable energy were, with cornmeal at 61, patent flour 53, low-grade flour 57, wheat brown shorts 41 and wheat bran 41. When measured by gains of energy in protein and fat of growing chickens, neither the total nutrients, the digestible nutrients, nor the metabolizable energy are correct measures of the feeding values of cornmeal, wheat flour, wheat brown shorts and wheat bran.

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THE EFFECTS PRODUCED BY AN INCREASE IN THE CALCIUM AND PHOSPHORUS CONTENT OF THE DIET ON THE CALCIUM AND PHOSPHORUS BALANCE AND ON VARIOUS BODILY CONSTITUENTS OF THE RAT [1]

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An excess of inorganic phosphate in the diet of albino rats in the form of orthophosphoric acid, or as the acid, basic or neutral phosphate of sodium or potassium has been shown by MacKay and Oliver ('35) to result in pronounced renal lesions. Calcification of a necrotic debris that fills the tubules presented a striking feature of the lesions. These effects on the kidney which we have confirmed in preliminary experiments, suggested the present investigation in which the effects of a large intake of calcium and phosphorus on the deposition of these elements and on the weight, water, protein and fat content of the body were determined.

PROCEDURE

The animals used in these experiments were the offspring of inbred Wistar rats and were selected in groups of three litter mates of approximately the same weight. Seven groups of males and an equal number of females, or a total of forty-two animals, were used. When weaned at the age of 21 days, they were placed in individual cages and fed the Wistar diet for 7 days. During this period the food intake was controlled so that each animal consumed the same amount of food as its

[1] Presented before the American Physiological Society at the Toronto meeting, April 26–29, 1939.

litter mates. The animals were then placed on the experimental rations. The basal ration consisted of 68% sucrose, 18% casein, 10% yeast and 4% of the Osborne and Mendel salt mixture. Sufficient $CaCO_3$ was added to give a Ca/P ratio of 2:1, as determined by analysis of the mixture; without this addition the ratio was 1:1. One animal in each group was fed the basal ration to serve as a control; the other two in the group were fed the same ration with additional amounts of calcium and phosphorus. Phosphorus was added in the form of a neutral mixture of mono- and dibasic sodium phosphate in sufficient quantity to increase the phosphorus content to approximately 2.5 and 3.2 times the amount present in the basal diet. Calcium was added as $CaCO_3$ in amounts sufficient to maintain the Ca/P ratio within the range of 1.6 to 2.0. Each animal was given daily by medicine dropper, 7 drops of a 6:1 mixture of cod liver oil and linoleic acid.

The basal ration will hereafter be referred to as ration 1 and those with the larger amounts of phosphorus and calcium as rations 2 and 3, respectively. Preliminary experiments showed that on rations 2 and 3 the kidney lesions described by MacKay and Oliver occurred invariably whereas the kidneys of the control animals on ration 1 were normal.[2]

The animals were fed on the experimental rations for 10 weeks, by the paired feeding technic of Mitchell and Beadles ('30). The food intake of litter mates was kept approximately the same from day to day and exactly equalized once a week. In making this equalization the actual bulk of the food mixture consumed by the animals on rations 2 and 3, owing to the higher salt content, had to be larger than that of the controls in order that the caloric value would remain the same. The feces were collected over the entire period of the experiment including the preliminary week on the Wistar diet. The bottom of the cages was constructed of wire mesh sufficiently large to permit free passage of the feces on to a

[2] Histopathological examination of the kidneys was made by Dr. Everett Bishop, pathologist to the Steiner and the Winship Clinics, Atlanta, Georgia.

fine mesh screen beneath. The collected feces were dried, weighed and analyzed for calcium and phosphorus.

At the conclusion of the experiment the animals were fasted for 24 hours with free access to water, and then killed by a blow on the head. The bodies were chopped with a meat cleaver, ground in a sausage grinder and finally in a corn mill. The intestinal contents were not removed. This procedure produced a homogeneous mass as shown by checks obtained on analyses of several aliquots for calcium, phosphorus, fat, nitrogen and water.

The analytical procedure was as follows: An aliquot of approximately 20 gm. was boiled in 30% KOH and after boiling, one sample taken for the analysis of fat by the method of Leathes and Raper ('25) as described by Feyder ('35) and another for the determination of nitrogen by the macro-Kjeldahl method. The determinations of calcium and phosphorus were made on separate aliquots of tissue which were ashed by the usual procedure and dissolved in HCl. Phosphorus was determined by the method of the Association of Official Agricultural Chemists ('25) which was found to give approximately the same values as the Youngburg and Youngburg method ('30). Calcium was determined by the method of McCrudden as described by Peters and Van Slyke ('25). The water content of the body was determined by drying aliquots of ground tissue to constant weight over P_2O_5 in an evacuated desiccator. Loss of water in preparing the tissues was reduced to a minimum by grinding the animals in a room saturated with water vapor.

RESULTS

The data on the calcium and phosphorus balance are given in table 1 and the results of the analyses of the rats in table 2. Each value in the tables is an average obtained from seven experiments except where the males and females have been combined, in which case the averages were derived from fourteen experiments. As a general observation it may be stated that there were no sex differences discernible in the

TABLE 1

Calcium and phosphorus balance with different calcium and phosphorus intake

RATION [1]	NUMBER OF EXPERIMENTS	CALCIUM INTAKE	CALCIUM IN FECES	DIFFERENCE BETWEEN INTAKE AND OUTPUT	INCREASE IN ABSORPTION [2] OVER CONTROLS	PHOSPHORUS INTAKE	PHOSPHORUS IN FECES	DIFFERENCE BETWEEN INTAKE AND OUTPUT	INCREASE IN ABSORPTION [2] OVER CONTROLS
				Males					
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	7	7.53	5.26	2.27	..	3.87	2.04	1.83	..
2	7	15.12	12.46	2.66	0.39	9.64	3.99	5.65	3.82
3	7	19.17	15.80	3.37	1.10	12.56	5.74	6.82	4.99
				Females					
1	7	6.76	4.66	2.10	..	3.48	1.88	1.60	..
2	7	12.99	10.70	2.29	0.19	8.51	3.68	4.83	3.23
3	7	16.34	13.85	2.49	0.39	11.08	5.01	6.07	4.47
				Males and females					
1	14	7.14	4.96	2.18	..	3.67	1.96	1.71	..
2	14	14.06	11.58	2.48	0.30	9.07	3.84	5.23	3.52
3	14	17.76	14.83	2.93	0.75	11.82	5.37	6.45	4.74

[1] Ration no. 1 contained the Osborne-Mendel salt mixture; nos. 2 and 3, 2 and 2.5 times, respectively, as much calcium and 2.5 and 3.2 times as much phosphorus as no. 1.

[2] The 'difference' in the preceding column is regarded for convenience as the amount absorbed.

results obtained from feeding the different rations except in body weight.

A statistical analysis of the body constituents is presented in table 3. The significance of the average difference between litter mates was determined by calculating the value of t according to Fisher's procedure ('28). To allow for the correlation between litter mates, t was determined by the formula on page 112 of Fisher's manual. Since the various differences between the animals on the control ration and those on rations 2 and 3 were of the same order for the males as for the females, the statistical analysis was made on the combined data for the two sexes. The probabilities of the differences obtained with different rations being a chance occurrence will hereafter be referred to as P. The values of P in the case of the body constituents are given in table 3.

The intake of calcium and phosphorus subtracted from the amount found in the feces will be referred to for convenience as the amount absorbed. It is possible that a larger amount may have been absorbed than estimated in this way, which in turn was balanced by a correspondingly larger excretion by the intestinal wall, but for present descriptive purposes it is unnecessary to take this possibility into account. With an increase in the intake of approximately 7 and 10 gm., respectively, the animals on rations 2 and 3 absorbed only 0.3 and 0.75 gm. more than those on the control diet. These increases in absorption though small were definitely significant in both cases as P was equal to 0.015 in the first instance and was less than 0.0003 in the second. With the small increase in calcium absorption on ration 2 there was a very small increase in the deposition of calcium which was not significant. On ration 3, however, with a slightly larger increase in calcium absorption there was also a slightly larger increase in deposition which, although small in absolute amount, was nevertheless significant. These results are in keeping with those of Whitcher, Booher and Sherman ('36) who found that with a phosphorus content of 0.42 to 0.43% in the diet, a graded increase in calcium from 0.43 to 1.04% did not bring about any

distinct increase in the rate of calcification in the body, but when the phosphorus content was raised to 0.73% graded increases in calcium to 1.05% induced very small increases in calcification. In our experiments the calcium content of the control ration was 1.1% and of rations 2 and 3, 2.1 and 2.6%; the phosphorus content of rations 2 and 3 was increased from 0.5% in the control ration to 1.3 and 1.7%.

Absorption of phosphorus on the different rations presents an entirely different picture than the absorption of calcium. On rations 2 and 3 with an increase of approximately 5.4 and 8.0 gm. in the phosphorus intake there was a definite increase in absorption of approximately 3.5 and 4.7 gm. (table 1). Notwithstanding this large increase in the absorption of phosphorus there was no significant change in the amount deposited in the body. Owing to the slight increase in calcium deposition without a corresponding increase in phosphorus, there was a slight rise in the Ca/P ratio of the body from 2.0 to 2.1.

The percentage gain in the body weight taken after a 24-hour fast immediately before the animals were sacrificed is given in table 2. The males gained 436, 445 and 418% over their original weight on rations 1, 2 and 3, respectively, and the females 326, 336 and 323%. This order of percentage gains was the same when the weights were taken before the fast. The small differences that were found between the groups of litter mates was in no instance significant. Conclusions drawn from these data, however, with regard to the effect of high calcium-phosphorus intake on body weight would be misleading for, as will be observed in table 2, the bodies of the animals on the high calcium-phosphorus diets contained a higher percentage of water and consequently a smaller amount of solid material than their controls. The average dry weight of the controls (males and females combined) in absolute amounts was 4.9 and 11.2 gm. more than that of their litter mates on rations 2 and 3, respectively, the differences being definitely significant (table 3). In this connection it is of interest to note that the average water

TABLE 2

Composition of rats with different calcium and phosphorus intake

RATION [1]	NUMBER OF EXPERIMENTS	INITIAL WEIGHT	FINAL WEIGHT AFTER 24-HOUR FAST	INCREASE OVER INITIAL WEIGHT	WATER CONTENT	DRY WEIGHT	FAT	PROTEIN (N × 6.25)	CALCIUM	PHOSPHORUS	Ca/P RATIO
Males											
		gm.	*gm.*	%	%	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
1	7	39	209	436	58.7	86.6	33.4	38.5	2.47	1.27	1.9
2	7	38	207	445	60.9	81.0	28.2	37.7	2.55	1.24	2.1
3	7	39	202	418	63.0	75.0	21.8	39.6	2.74	1.31	2.1
Females											
1	7	38	162	326	59.8	65.0	22.8	29.8	2.30	1.10	2.1
2	7	36	157	336	61.4	60.8	19.6	29.2	2.31	1.11	2.1
3	7	35	148	323	63.6	54.2	14.2	28.3	2.34	1.12	2.1
Males and females											
1	14	39	186	377	59.2	75.8	28.1	34.2	2.39	1.19	2.0
2	14	37	182	391	61.2	70.9	23.9	33.5	2.43	1.18	2.1
3	14	37	175	376	63.3	64.6	18.0	34.0	2.54	1.22	2.1

[1] Ration no. 1 contained the Osborne-Mendel salt mixture; nos. 2 and 3, 2 and 2.5 times, respectively, as much calcium and 2.5 and 3.2 times as much phosphorus as no. 1.

intake over the entire experiment on the three rations was 1.17, 1.64, and 2.53 liters, respectively.

The total amount of body protein was slightly different on the three rations but these differences, as shown in table 3, were not significant. The fat content, however, paralleling the decrease in dry weight, was significantly smaller with the larger intake of calcium and phosphorus. On the control ration the bodies contained 4.2 and 10.1 gm. more fat than on

TABLE 3

Statistical analysis

Comparison of body constituents on rations 1 and 2

	CALCIUM	PHOSPHORUS	DRY WEIGHT	FAT	PROTEIN
Mean difference[1]	+0.06	−0.010	− 4.9	− 4.2	−0.7
Standard deviation	0.149	0.166	4.62	4.8	2.3
Probability	0.084	0.43	0.001	0.003	0.14
Comparison of body constituents on rations 1 and 3					
Mean difference[2]	+0.17	+0.03	−11.2	−10.1	−0.2
Standard deviation	0.219	0.104	4.6	4.32	3.1
Probability	0.008	0.15	< 0.0001	< 0.0001	0.39
Comparison of body constituents on rations 2 and 3					
Mean difference[2]	+0.11	+0.04	− 6.3	− 5.9	+0.5
Standard deviation	0.226	0.107	6.2	4.1	2.4
Probability	0.052	0.10	0.001	0.0001	0.23

[1] A + sign indicates a greater average content on ration 2; a — sign the reverse.
[2] A + sign indicates a greater average content on ration 3; a — sign the reverse.

rations 2 and 3 which was practically the same as the amounts by which the controls exceeded the other animals in dry weight, namely, 4.9 and 11.2 gm., respectively. It may be concluded therefore that the smaller dry weight was due to a diminution in the formation or deposition of fat.

DISCUSSION

Since a portion of the calcium which was absorbed in rations 2 and 3 in excess of the amount absorbed in the control ration was deposited in the body, the remainder of the excess

absorbed, or 0.26 and 0.60 gm., respectively, (combined averages of males and females) must have been excreted in the urine. In the case of phosphorus, with a large increase in absorption and no increase in deposition, all the excess absorbed which amounted to 3.52 and 4.74 gm. must have been excreted.

Collection of the urine for analysis of calcium and phosphorus presented insurmountable difficulties inasmuch as the small amount of food mixture invariably spilt by the animals would have dropped into the urine thereby vitiating the results. An approximation, however, of the total urinary excretion of calcium and phosphorus on the three rations throughout the experiment can be obtained by a few simple calculations. From an analysis of three weanling male rats and three females of the same strain and approximately the same average weight as of those used in these experiments, it was found that their bodies contained 0.28 gm. calcium and 0.18 gm. phosphorus. Subtraction of these values from the total calcium and phosphorus found in the animals at the conclusion of the experiment gave 2.11, 2.15 and 2.26 gm. calcium respectively, deposited by the animals on the three rations and 1.01, 1.00 and 1.04 gm. phosphorus. The difference between the amount deposited and that absorbed (table 1) may be taken as the amount excreted in the urine. In the case of calcium this was found to be 0.07, 0.33 and 0.67 gm. respectively on the three rations and in the case of phosphorus 0.70, 4.23 and 5.41 gm. There was therefore a marked difference between the urinary excretion of calcium and phosphorus. The average daily excretion of calcium was 4 and 8 mg., respectively, on rations 2 and 3 as compared with 1 mg. on the control ration, while the daily phosphorus excretion on rations 2 and 3 rose from an average of 90 mg. on the control ration to 500 and 675 mg., respectively. While these figures give an estimate of the relative amounts of calcium and phosphorus excreted daily by the kidneys, it should be noted that the actual daily excretion probably varied considerably from the averages

because of the variations at different periods of the experiment in the intake and rate of deposition in the body.

The smaller amount of fat in the animals receiving a larger amount of the calcium and phosphorus than the controls was probably due in small part to an interference in absorption of the food material. An estimate of the amount of food that remained unabsorbed was obtained by subtracting the ash content of the feces from the total dry weight. In the case of the controls this amounted to an average of 30.7 gm. and for the animals on rations 2 and 3, 35.6 and 37.2 gm., respectively, or 4.9 and 6.5 gm. more than on the control ration. From the formulae proposed for the conversion of carbohydrate into body fat (compare Rapport, '30), it can be estimated that the food mixture that escaped absorption on rations 2 and 3 would have yielded less than 2 and 2.5 gm. fat, respectively, whereas the body fat on these two rations was 4.2 and 10.1 less than on the control ration. The major portion of the differences in the fat content of the animals on the three rations must therefore have been due to factors other than differences in absorption of foodstuff.

SUMMARY AND CONCLUSIONS

When large amounts of calcium and phosphorus were added to the rations of albino rats practically all the excess calcium was recovered in the feces, whereas the phosphorus in the feces was considerably less than the excess intake. The kidneys were therefore called upon to excrete very little more calcium but much more phosphorus than on the control ration.

A small portion of the excess of absorbed calcium but none of the phosphorus was stored in the body.

The percentage gain in body weight was not affected by the high calcium-phosphorus intake. The dry weight of the body, however, was definitely lower.

The fat content of the body was significantly reduced by the increased intake of calcium and phosphorus. The diminution in the total fat and dry weight was approximately the same.

Both the fat and dry weight of the body showed a progressive decline with a progressive increase in the calcium-phosphorus intake.

The large amount of calcium and phosphorus interfered to a slight extent with the absorption of food material as shown by combustion of the feces. The effect on absorption, however, could account only in a small degree for the diminution in the fat content of the body.

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SIMULTANEOUS APPEARANCE OF A POSITIVE LINE TEST AND RADIOACTIVE PHOSPHATE DEPOSITION IN THE RACHITIC RAT METAPHYSIS [1]

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FOUR FIGURES AND ONE PLATE (FIVE FIGURES)

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Artificially produced radioactive isotopes of several physiologically important elements are proving of great value in studies of intermediary metabolism. This is especially true of the unstable isotope of phosphorus (P^{32}) as applied to studies of the calcification processes. Due to the high degree of biological standardization which has been achieved in the technic of assay for vitamin D, the rachitic rat, as prepared for this purpose, offers a very satisfactory tool for the investigation of vitamin D action as traced by radioactive phosphorus. Within the past year several preliminary reports of investigations of this type have appeared. Dols and his associates ('37, '38 and '38 a) at Amsterdam have reported that vitamin D appears to have no characteristic mode of action on the absorption or re-excretion of administered phosphorus in the gut of the rachitic rat. Neither were they able to detect any difference between normal and rachitic rats in the rate

[1] These data are taken from a portion of the material presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Rochester, by Marian L. Manly.

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of synthesis of phospholipid. Using rachitic chicks, they have found that the phosphorus metabolism of the bone as a whole is more intense than that of normal birds. Furthermore, the radioactive phosphorus concentrated more rapidly in the epiphyses than in the diaphyses of the same bones. Very recently, Cohn and Greenberg ('39) have reported, in abstract form, the results of experiments on rats from which they concluded: "The influence of vitamin D on the deposition of inorganic phosphorus in the bone of rachitic rats must be ascribed to a more direct effect than one merely resulting from an increased absorption. A specific effect of vitamin D on organic bone phosphorus and a specific role of this fraction in bone formation are indicated."

This paper constitutes one of a series from this laboratory employing radioactive phosphorus (P^{32}) for the investigation of problems relating to the calcification of bones and teeth.

EXPERIMENTAL

Radioactive sodium phosphate.[4] A sample of Na_2HPO_4 containing radioactive phosphorus was dissolved in distilled water and made up to a convenient volume from which aliquots were taken for further dilution to concentrations suitable for administration to rats.

Plan of procedure. Two series of young rachitic rats from our standardized vitamin D assay colony were used, the first consisting of 18, the second of 38 animals, making a total of 56. They were distributed among the experimental and control groups as shown in table 1. All animals received a single dose each of radioactive phosphorus (as Na_2HPO_4) in 1.0 cc. of water by stomach tube. In series 1, the dose of phosphorus was 0.1 mg. and yielded 96,000 c.p.m.[5] at the time administered. In series 2, 0.2 mg. of phosphorus yielded 80,000 c.p.m. Vitamin D, as crystalline D_2 dissolved in propylene glycol, was also given by stomach tube at the same time as the phos-

[4] The authors are indebted for this material to the Radiation Laboratory of the University of California.

[5] Counts per minute (c.p.m.) as determined by our scale-of-four counters.

phorus. The amount of glycol solution was 0.2 cc. per rat and for series 1 contained 10 I.U. and for series 2, 15 I.U. per dose. At the time of the experiment, the animals averaged 80 gm. in weight and 50 days of age and had been prepared by a period of 23 days on the rachitogenic diet 2965 of Steenbock and Black ('25).

Samples. At various times after dosing, as indicated in table 1, the animals were sacrificed by decapitation and the

TABLE 1

Deposition of radioactive phosphate in the rachitic rat metaphysis

TIME	NUMBER OF RATS	DOSE P AS Na_2HPO_4	RADIO-ACTIVITY COUNTS PER MINUTE	DOSE OF VITAMIN D	AVERAGE FRACTION TOTAL RADIOACTIVITY PER GRAM OF FRESH TISSUE			LINE TEST
					Blood	Diaphyses	Metaphyses	
hours		mg.		I.U.	%	%	%	
				Series 1				
2	4	0.1	96,000	10	0.15	1.31	0.10	
4	2	0.1	96,000	10	0.25	1.58	0.21	
8	3	0.1	96,000	10	0.29	2.26	0.33	
12	2	0.1	96,000	10	0.27	2.27	0.29	
24	3	0.1	96,000	10	0.24	3.10	0.38	
92	2	0.1	96,000	10	0.22	3.46	1.71	
92	2	0.1	96,000	None	0.17	3.10	0.57	
				Series 2				
2	3	0.2	80,000	15	0.08	0.81	0.09	—
2	1	0.2	80,000	None	0.19	2.03	0.20	—
5	3	0.2	80,000	15	0.18	1.68	0.35	—
5	2	0.2	80,000	None	0.18	1.70	0.28	—
8	3	0.2	80,000	15	0.15	1.23	0.18	—
8	2	0.2	80,000	None	0.21	2.11	0.29	—
12	3	0.2	80,000	15	0.18	1.53	0.17	—
12	2	0.2	80,000	None	0.25	2.09	0.38	—
24	3	0.2	80,000	15	0.31	2.71	0.62	—
24	2	0.2	80,000	None	0.27	1.90	0.50	—
54	3	0.2	80,000	15	0.21	2.01	0.63	±
54	2	0.2	80,000	None	0.19	2.30	0.42	—
72	2	0.2	80,000	15	0.22	3.19	2.46	+
72	2	0.2	80,000	None	0.24	2.79	0.66	—
96	3	0.2	80,000	15	0.22	2.31	2.31	++
96	2	0.2	80,000	None	0.15	2.50	0.55	—

blood collected in a flask containing 0.2 cc. of saturated sodium citrate solution and weighed. Both tibias (with fibulas attached) were dissected out and slabs 1.5 to 2.0 mm. thick were sectioned longitudinally from the center of the bones in the plane of attachment between the tibia and fibula. The slabs were then stained with silver nitrate according to the standard line test procedure and photographed. With the micro-jigsaw apparatus of Deakins and Manly ('39), a sample was cut from the metaphysis to include the area of new calcifica-

Fig. 1 Diagrammatic drawing of tibial slab. A, B and C are epiphysis, metaphysis and diaphysis, respectively. D represents the area of new calcification shown by silver nitrate staining. The metaphyseal area within the dotted line was that cut out by the micro-jigsaw for determination of radioactive phosphorus content of newly deposited bone salt.

tion (the 'line') as illustrated by the drawing in figure 1. The weight of these blocks of metaphyseal cartilage averaged 12 to 13 mg. Samples of tibial diaphysis (including the marrow) were also taken. The two samples of metaphysis and diaphysis from each rat were pooled and weighed. All samples, including blood, were dried on a hot plate, ashed in an electric oven at 500°C. over night, the ash dissolved in concentrated HNO_3 and HCl, made up to 2.0 cc. volume and the radioactivity determined with a scale-of-four Geiger-Müller counter (Bale, Haven and LeFevre, '39). The phosphorus in

the doses of Na_2HPO_4 solution was determined in duplicate by the method of Holtz ('29).

RESULTS

To allow for small differences in body weight of the rats, all of the results were multiplied by the body weight and divided by 80 gm. (the approximate average of the group). Hence, the data are expressed as per cent of the total dose of radioactive P per gram of fresh tissue per 80 gm. rat. The average values for the rats of each time period for the three tissues studied are to be found in table 1. The data are also expressed graphically in figures 2, 3 and 4.

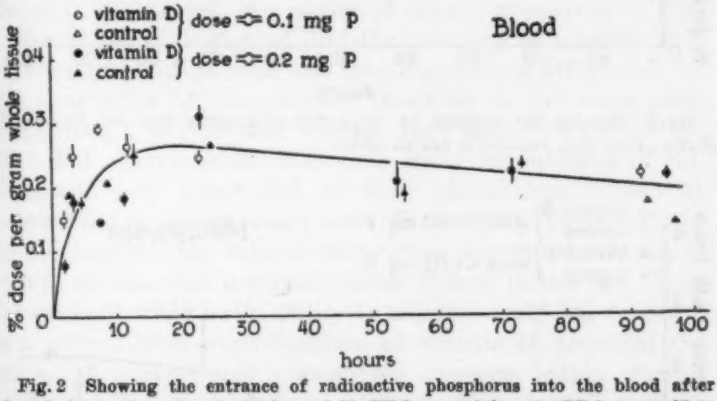

Fig. 2 Showing the entrance of radioactive phosphorus into the blood after the administration of a single dose of Na_2HPO_4 containing the P^{32} isotope. Note that vitamin D has no effect on the values found after either 0.1 or 0.2 mg. of P.

Blood. The radioactive phosphorus in the blood (fig. 2) reaches a maximum of 0.27% in less than 24 hours and decreases slowly to 0.20% in 4 days. There is no apparent difference between the amount of radioactive phosphorus in the blood of the rats fed vitamin D and that of the controls.

Diaphyses. The radioactive phosphorus in the diaphyses (fig. 3) rises slowly to 2.5% in 24 hours and continues to rise throughout the experimental period. Again, there is no apparent difference between vitamin D-fed rats and controls.

Metaphyses. The values for the vitamin D rats and the controls both fall along the same line at 0.5% up to 54 hours (fig. 4). After 54 hours, however, the values for the vitamin

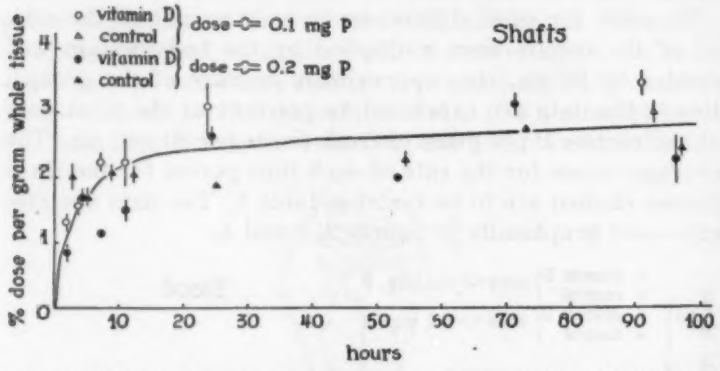

Fig. 3 Showing the entrance of radioactive phosphorus into the diaphyseal shafts. Here also, vitamin D has no effect.

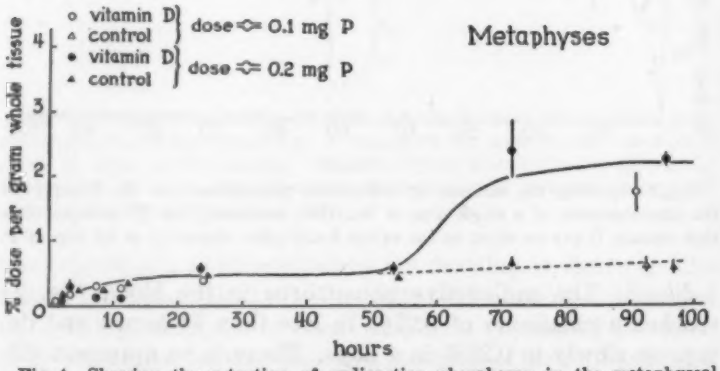

Fig. 4 Showing the retention of radioactive phosphorus in the metaphyseal cartilage. Note that at 72 hours the values in the vitamin D fed rats are significantly higher than those of the controls.

D-fed rats rise above 2.0% while the controls remain at the 0.5% level. The average degree of healing produced by vitamin D in series 2 is indicated in the last column of table 1.

These results show that the development of the 'line' of new calcification in the rachitic metaphysis occurs concomitantly with the entrance of the radioactive phosphorus into this area.

DISCUSSION

Almost nothing is known concerning the metabolic processes by means of which vitamin D mediates the formation of new bone salt in the rachitic animal. Various theories have been proposed among which that of Harris and his associates ('31 and '32) has probably been most widely cited. These workers believed that the chief function of vitamin D is to increase the "net absorption of calcium and/or phosphorus" from the gut. Nicolaysen, in a series of papers appearing in 1937 ('36 and '37 a), indicated that the influence of vitamin D is on absorption of calcium and that it has very little effect on the absorption of phosphorus. However, in the same year, Nicolaysen ('37) further showed that rachitic rats on the 2965 diet absorb about twice their daily requirement of calcium and only about 30% of their phosphorus needs. As pointed out by Morgareidge and O'Brien ('38), these findings do not support the calcium absorption theory of vitamin D action, at least for low-phosphorus rickets in the rat. That the rat is peculiar in its ability to adjust its mineral economy to a normal level when deprived of vitamin D, provided the intake of calcium and phosphorus remains within normal limits, is a fact that has been emphasized by many investigators. Nevertheless, O'Brien and Morgareidge ('39) have shown that small doses of vitamin D are very effective in increasing the efficiency with which additional phosphorus is utilized by the rachitic rat on the 2965 diet. If, therefore, the observations of Nicolaysen and others are correct that phosphorus absorption from the gut is independent of vitamin D, the conclusion that this vitamin plays an important role in the intermediary metabolism of the bone forming elements must be seriously considered. In this connection, we feel that the experiments with radioactive phosphorus reported in the present paper are significant.

In interpreting these results, it is to be emphasized that the total amount of phosphorus actually administered was only 0.1 to 0.2 mg., an amount too small to constitute a factor per se in the healing of rickets, yet, the high content of the radioactive isotope in the sample (80,000 c.p.m. per dose) made the tracing of the tagged phosphorus relatively easy.

Of major interest is the finding that vitamin D had no effect on the entrance of phosphorus into the blood or into the tibial diaphysis. The values found are in general agreement with those previously reported from this laboratory (Manly and Bale, '39). In the rachitic metaphyses, however, the effect of vitamin D is clearly seen. The entrance of phosphorus into this structure corresponds almost exactly with the appearance of the 'line' produced by silver nitrate staining in those animals which had received a single dose of the vitamin some 54 or more hours previously. In the metaphyses of the control animals the 'line' did not appear nor did the content of radioactive phosphorus increase. We feel that such evidence lends definite support to the conclusion that vitamin D has for at least one of its actions, a direct influence on the metabolic factors controlling calcification. This is in contradistinction to any possible role it may play in intestinal absorption.

The fact that from 54 to 72 hours must elapse between administering the vitamin and the entrance of phosphorus into the fixed bone salt which is responsible for the line test may be taken as additional evidence that mere absorption is not the limiting factor. Kramer, Shear and Siegel ('31) showed that simple addition of phosphorus to the 2965 diet may result in the inception of healing in rachitic rats in as short a time as 12 hours. The inference is, therefore, that under the conditions described here, the time interval between administration of the vitamin and the beginning of new calcification is that required for the metabolic action of vitamin D to mobilize the phosphorus required for the healing process.

SUMMARY

Following a single dose of Na_2HPO_4 containing the radioactive isotope (P^{32}) accompanied by a single dose of vitamin D_2, determinations have been made of the radioactive phosphorus content of whole blood and of the tibial diaphysis and metaphysis of rachitic rats. The data show that vitamin D has no influence on the entrance of phosphorus into the blood or into the diaphyseal portion of the tibia. In the metaphysis, however, the healing produced by the vitamin (as shown by the appearance of a positive line test) occurs concomitantly with a significant increase in the content of radioactive phosphorus. These effects in the metaphysis are seen only after the elapse of 54 to 72 hours subsequent to the administration of the vitamin. It is pointed out that the results support the conclusion that the mode of action of vitamin D is not limited to the control of intestinal absorption of the elements concerned in calcification.

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PLATE 1

EXPLANATION OF FIGURES

1, 2, and 3 Left and right tibial slabs stained with silver nitrate 96 hours after the administration of 15 I.U. of vitamin D_3 and 0.2 mg. of phosphorus (as Na_2HPO_4) containing radioactive P^{32} corresponding to a total of 80,000 c.p.m. Per cent of total radioactivity per gram of fresh metaphyseal cartilage was 1.2, 2.6 and 3.1, respectively, for the three rats. The intensity of the line of new calcification increases with increasing content of the radioactive isotope.

4 and 5 Tibial slabs from two 96-hour control rats which received the same dose of phosphorus but no vitamin D. Note absence of healing. Per cent of total dose of radioactivity per gram of metaphyseal cartilage in these two rats was 0.75 and 0.35, respectively.

THE EFFECT OF HEAT AND SOLVENTS ON THE NUTRITIVE VALUE OF SOYBEAN PROTEIN [1]

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Reviewing the literature on the effect of heat on the nutritive value of proteins, Morris ('37) made the statement that "in view of the mass of contradictory evidence further confirmation of the work of Hayward seems essential." Morris was referring to the observations made on meat protein (Morgan and Kern, '34), casein (Greaves and Morgan, '34; Block, Jones and Gersdorff, '34), and skim milk powders (Fairbanks and Mitchell, '35) confirmed by other studies which revealed that the nutritive value of the proteins had been lowered by heat. This was in contrast to the demonstration of Hayward, Steenbock and Bohstedt ('36 a) that the proteins of expeller and hydraulic soybean oil meals prepared at high temperatures were nutritionally superior to those prepared at low temperatures. Later Wilgus, Norris and Heuser ('36) working with chicks at Cornell reported that solvent soybean oil meal was superior to the high temperature expeller and hydraulic soybean oil meals. They suggested that since the temperature used in the solvent process was considerably lower than that used in the preparation of the expeller and hydraulic meals, a factor other than temperature was responsible for the difference.

The question arose as to whether treatment with certain fat solvents could alter the protein molecule of the soybean

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

so as to make it more available inasmuch as results obtained by Mashino ('29) and Kajizuka ('35) had suggested such a possibility.

Other investigators have given consideration to the removal of hypothetical toxic factors by solvents or their destruction by heat. Osborne and Mendel ('17), who studied the nutritive value of soybeans as early as 1917, stated that "evidently there is nothing toxic in the raw meal, for none of the rats which ate it died." Waterman and Johns ('21) reported that the increased digestion of phaseolin after heating was due to the "loss of a protein toxicity or the destruction of an associated toxic substance." Shrewsbury and co-workers ('32) observed that cooking increased the digestibility of the soybeans only slightly. Their theory was that heating caused the removal or destruction of certain materials of a toxic nature.

Since Hayward, Steenbock and Bohstedt ('36 a and b) had shown that heated soybeans had a higher biological value than raw soybeans and that cystine additions to raw soybeans increased the amount of nitrogen retained, it appeared desirable to obtain data on sulfur balances as well.

EXPERIMENTAL

The soybeans used were of the Illini variety harvested in 1934. From these special expeller soybean meals were prepared in commercial plants and autoclaved and solvent treated samples were prepared in the laboratory. The expeller soybean meal used for our feeding trials had been exposed to the comparatively high temperature of 150°C. This was chosen in preference to others prepared at a lower temperature since soybean meal processed at such a high temperature had been found to have the highest biological value (Hayward, Steenbock and Bohstedt, '36 a). The autoclaved soybean preparations were prepared as described by Hayward, Steenbock and Bohstedt ('36 a) by autoclaving them for 1½ hours at 17 pounds pressure.

The solvents used for the extraction of the soybeans were commercial petroleum ether, hexane and a mixture of ninety parts of hexane and ten parts of methanol. The solvent treated soybeans were prepared as follows: ground soybean and solvent were poured alternately into a percolator and then stirred. Small amounts of each were added until 2 kg. of soybeans had been used. The extraction was continued over night at room temperature. The solvent was then drained off slowly in 3 to 4 hours. Four extractions were made. For the first two extractions 2 to 2½ liters of solvent were used; for the later extractions 1 to 1½ liters.

The soybean residue was freed from most of the solvent in 2 hours with frequent stirring before an electric fan. Finally it was exposed in shallow trays to the air over night. The solvent was removed from the oil at room temperature with nitrogen under reduced pressure.

The soybean residues and oils were fed in the following combinations: a) residue plus oil prepared at room temperature, and b) residue and oil autoclaved together at 17 pounds pressure for 1½ hours. The composition of the rations in which these were fed was as follows: solvent treated soybeans 46 parts, cod liver oil 2 parts, salt mixture (Steenbock and Nelson, '23) 4 parts and cooked starch 48 parts. The raw or autoclaved soybeans were fed in the following ration: soybeans 45, cod liver oil 2, salt mixture 4 and cooked starch 49. In the soybean oil meal ration thirty-eight parts of the meal were mixed with eight parts of soybean oil so as to equal the ratio in which they occur in the raw soybean. The protein was fed at a level of 18%. To insure that most of the sulfur came from the soybeans, sulfates were omitted from the salt mixture.

Routine care of animals

The daily food allowances, weighed to centigrams, were fed to the rats at approximately the same time of day. The maximum level of equalized food intake was determined by a preliminary feeding period of 3 to 5 days. During the experi-

mental periods of 4 to 7 days all rats in a group consumed the same amount of food. They were weighed at the beginning and end of each preliminary and experimental period.

Description of metabolism cages and care of excreta

The rats were quartered in individual metabolism cages of glass cylinders, 9 inches in diameter, resting on screens over glass funnels, 10 inches in diameter and 14 inches high. The separation of the feces from the urine was accomplished by allowing the feces to collect in an antiseptic chamber containing blotting paper soaked in formaldehyde as the urine flowed over a glass bulb through a small funnel to a collecting flask containing toluene. The receivers were emptied daily into storage flasks and rinsed with distilled water. In the experiments for the determination of sulfur distribution in the urine, the filtered urine preserved with toluene was stored at 5°C. In groups 1 and 2 sulfur was determined on the urine of each rat for each period (table 2). In the other groups it was determined on composite samples from four rats for each period (table 1).

To prevent loss of sulfur the feces were treated with 10 cc. of 2% copper acetate (Cuthbertson and Turnbull, '34) in 50 cc. Erlenmeyer flasks. They were then dried first on a sand bath at 80 to 100°C. and then in an oven at 100°C. until constant in weight. Analyses for sulfur were made on ground samples composited the same as the urine samples.

When nitrogen was determined on the urine and the feces, 10 cc. of 2% H_3PO_4 were added to the storage flasks. Analyses for nitrogen were made on separate composites of urine and of feces from the four rats in the group for each period.

Methods of analyses

Total sulfur of the rations and feces was determined by sodium peroxide fusion. The fat of the ration was extracted with anhydrous ether before analysis to prevent foaming during fusion. This was permissible because analysis of soy-

TABLE 1
Sulfur absorbed and retained on heated versus raw soybean diets

TABLE 1

Sulfur absorbed and retained on heated versus raw soybean diets

GROUP [1]	PERIOD [2]	WEIGHT OF RATS AVERAGE	FOOD CONSUMED	SULFUR [6]							
				In ration		Excreted		Absorbed		Retained	
				Per cent	Weight	Feces	Urine	Weight	Per cent	Weight	Per cent
Soybean oil meal (expeller—150°C.)											
		gm.	*gm.*	%	*gm.*	*gm.*	*gm.*	*gm.*	%	*gm.*	%
1	1	97	96	0.165	0.159	0.060	0.058	0.099	62.3	0.041	41.4
2	2	111	86	0.160	0.137	0.048	0.062	0.089	64.2	0.027	30.3
Whole soybean, raw											
1	2	96	96	0.164	0.157	0.061	0.078	0.096	61.1	0.018	18.7
2	1	110	93	0.167	0.155	0.063	0.081	0.092	59.4	0.011	12.0
Whole soybean, autoclaved [3]											
3	1	128	200	0.168	0.336	0.129	0.116	0.207	61.6	0.091	44.0
4	1	124	207	0.168	0.348	0.131	0.120	0.217	62.4	0.097	44.7
Whole soybean, raw											
3	2	142	202	0.168	0.339	0.118	0.193	0.221	65.2	0.028	12.7
4	2	141	215	0.168	0.361	0.123	0.183	0.238	65.9	0.055	23.1
Ether extracted soybean plus oil											
5	1 [4]	117	217 (auto)	0.175	0.379	0.146	0.120	0.233	61.5	0.113	48.5
5	2 [5]	124	211 (raw)	0.177	0.373	0.143	0.164	0.230	61.7	0.066	28.7
Hexane extracted soybean plus oil											
6	1 [4]	97	173 (auto)	0.174	0.301	0.107	0.101	0.194	64.5	0.093	47.9
6	2 [5]	101	161 (raw)	0.166	0.267	0.102	0.137	0.165	61.8	0.028	17.0
Hexane-methanol extracted soybean plus oil											
7	1 [4]	93	173 (auto)	0.178	0.308	0.108	0.092	0.200	64.9	0.108	54.0
7	2 [5]	95	151 (raw)	0.172	0.261	0.090	0.144	0.171	65.5	0.027	15.8

[1] Each group had four male rats, ages ranging from 5 to 7 weeks.

[2] Period consisted of 4 days for groups 1 and 2; of 7 days for groups 3 to 7 inclusive.

[3] Soybeans were autoclaved at 17 pounds pressure for 1½ hours.

[4] Extracted soybean residue and oil autoclaved together 1½ hours at 17 pounds pressure.

[5] Extracted soybean residue and oil prepared at room temperature.

[6] In groups 1 and 2 sulfur was determined on the urine of each rat for each period (table 2). In the other groups sulfur determinations were made on composite samples of urine from four rats for each period. Analyses for sulfur of the feces were made on composite ground samples from the four rats in a group for each period. All figures represent the composites of four rats in a group for each period.

bean oil revealed the presence of only traces of sulfur, viz., 0.01%.

Total sulfur of the urine was determined by the Benedict-Denis method ('09, '10); and combined inorganic and ethereal sulfur by Folin's methods (Folin, '05, '06). Ethereal and neutral sulfur were obtained by difference.

Results

Table 1 contains the results of the sulfur balances of the heated and raw soybeans and of the soybeans treated with the various solvents. The sulfur was found to have been absorbed to the same degree on all the rations, with an average coefficient of digestibility of 63.0%. The retention of sulfur, on the contrary, varied greatly. The amount retained on the heated soybean rations, viz., commercial soybean meal and soybeans autoclaved in the laboratory ranged from 30.3 to 44.7% of the intake, the average being 40.1%; on the raw soybean rations, 12.0 to 23.1% with an average of 16.6%.

With the solvent-treated soybean prepared at room temperature, more sulfur was retained from rations in which the residues and oil from these had been autoclaved together than from rations in which they were fed together with no heat treatment. In the former the sulfur retention averaged 50.1%, in the latter 20.5. Apparently the solvent itself had no beneficial effect.

In table 2 are given the amounts of total sulfur excreted in the urine by individual rats for periods of 4 days. All rats eliminated less sulfur on the heated than on the raw soybean ration. Collectively, groups 1 and 2 of four animals each on raw soybeans excreted 34.5 and 30.6% more sulfur in the urine than those receiving the heated soybeans. For groups 3 to 7 inclusive the same relations are seen; a higher percentage of sulfur was excreted in the urine on the unheated than on the autoclaved soybeans (table 1). In table 3 are given the results on nitrogen balances. These show that on heated soybeans the rats retained about 2.5 times as much sulfur and about 1.8 times as much nitrogen as on the raw soybeans.

TABLE 2

Urinary sulfur of individual rats in 4-day periods

GROUP [1]	RAT	WEIGHT OF RATS	PERIOD OF 4 DAYS	SOYBEAN OIL MEAL (EXPELLER—150°C.)		PERIOD [2] OF 4 DAYS	WHOLE SOYBEAN, RAW	
				Food consumed	Sulfur in urine		Food consumed	Sulfur in urine
	no.	gm.	no.	gm.	gm.	no.	gm.	gm.
1	1	96	1	24	0.016	2	24	0.021
	2	97		24	0.013		24	0.018
	3	100		24	0.015		24	0.020
	4	95		24	0.014		24	0.019
Total				96	0.058		96	0.078
2	5	114	2	24	0.016	1	24	0.020
	6	102		19	0.013		22.5	0.020
	7	112		24	0.019		24	0.023
	8	112		19	0.014		22.5	0.018
Total				86	0.062		93	0.081

[1] In groups 1 and 2 sulfur was determined on the urine of each rat for each period of 4 days.

[2] The sequence of periods 1 and 2 was reversed for group 2.

TABLE 3

Sulfur and nitrogen absorbed and retained on heated versus raw soybean diets

Group 2

DIET	FOOD CONSUMED	IN RATION		EXCRETED		ABSORBED		RETAINED	
		Per cent	Weight	Feces	Urine	Weight	Per cent	Weight	Per cent
Sulfur [1]									
	gm.	%	gm.	gm.	gm.	gm.	%	gm.	%
Soybean oil meal (expeller—150°C.)	86	0.160	0.137	0.048	0.062	0.089	64.2	0.027	30.3
Whole soybean raw	93	0.167	0.155	0.063	0.081	0.092	59.4	0.011	12.0
Nitrogen [1]									
Soybean oil meal (expeller—150°C.)	86	2.57	2.20	0.455	1.172	1.745	79.3	0.573	32.8
Whole soybean raw	93	2.86	2.66	0.661	1.645	1.999	75.1	0.354	17.7

[1] All figures represent the composites of four rats in a group for a period of 4 days.

Since more urinary sulfur was excreted on the raw soybean ration than on the heated, the distribution of sulfur in the urine was of interest. If the sulfur-containing fraction of the protein of the raw soybean was not available because of its conjugation with some toxic factor, then it was possible that an increase in the neutral sulfur would have occurred with a corresponding decrease in the ethereal and inorganic sulfur forms. Four male rats 12 weeks of age and averaging 212 gm. in weight were used to determine this.

TABLE 4

Distribution of urinary sulfur on diets containing heated and raw soybeans

GROUP [1]	PERIOD [2]	WEIGHT OF RATS	FOOD CONSUMED	SULFUR [3]									
				In ration		Urine					Relation to total		
				Per cent	Weight	Total	Inorganic	Inorganic and ethereal	Ethereal	Neutral	Inorganic	Ethereal	Neutral
Whole soybean, raw													
		gm.	*gm.*	%	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%	%	%
8	1	224	126	0.167	0.210	0.159	0.103	0.119	0.016	0.040	64.8	10.1	25.1
Soybean oil meal (expeller—150°C.)													
8	2	212	129	0.160	0.206	0.133	0.082	0.097	0.015	0.036	62.3	11.3	26.4

[1] Group 8 had four male rats approximately 12 weeks of age.

[2] Period consisted of 5 days.

[3] All figures represent the composites of four rats in a group for each period.

The composition of the rations, the routine care of the animals, the metabolism cages and methods of analyses have already been described. Table 4 shows the inorganic, ethereal and neutral sulfur excreted in relation to the total. The results of the sulfur balance studies of these adult rats are not presented since the coefficients of digestibility on the heated and raw soybeans were similar to those of the young animals.

DISCUSSION

Some workers (Mitchell and Smuts, '32; Shrewsbury and Bratzler, '33) have contended that soybeans are deficient in cystine. They based their conclusion upon the observation

that ether extracted soybeans plus cystine produced a greater increase in the weight of animals than unsupplemented soybeans. Both the ether extracted soybeans and the unsupplemented soybeans were fed unheated.

Csonka and Jones ('34), on the other hand, stated that "The analytical data do not support the idea of a quantitative deficiency of cystine in soybean proteins, but they do admit the possibility of a qualitative deficiency due to low availability of amino acids when the raw meal is fed, particularly when soybeans containing the lower cystine values are used."

Osborne and Mendel ('17) pointed out that heat improved the nutritive value of the soybean. Extracting the soybean with ether failed to increase its biological value. Cooking the soybean made it more palatable than the raw. In this manner the increased food consumption resulted in a better gain. Vestal and Shrewsbury ('32) concluded from metabolism studies that improved growth on cooked or roasted soybeans was due to an increase in food consumption and a slight increase in the digestibility of nitrogen.

Hayward, Steenbock and Bohstedt ('36 a) found that cystine supplemented the raw soybean but failed to cause any improvement with the heated soybean. From both growth studies and nitrogen balance experiments it was concluded that heat was the factor which caused the protein to be well utilized by the animal.

In the present study the observation that heat improved the nutritive value of the soybean was confirmed. Sulfur metabolism studies showed clearly that the digestibility of the protein was approximately the same for the raw and heated soybean. The difference lay in the availability of the products of protein digestion. Less urinary sulfur was excreted on the heated soybean than on the raw, therefore more was retained.

The results on the soybeans treated with various solvents are contrary to those of others (Wilgus, Norris and Heuser, '36; Mashino, '29; Kajizuka, '35). These investigators be-

lieved that the action of the solvent itself was the factor which increased the biological value of the protein. However, the Cornell workers used commercial solvent soybean oil meal which had been exposed to a temperature of 98°C. for 15 minutes and to temperatures of 45 to 78°C. for an unspecified period. The Japanese workers did not report their temperatures. In our preparations the soybeans were extracted at room temperature, approximately 20 to 25°C., and the solvent was removed from the oil at the same temperature. Therefore, heat can be excluded as a determining factor in our laboratory preparations. Heat was applied only after the oil had been extracted from the soybean to permit comparing the effect of solvents alone and of solvents plus heat.

The percentage distribution of urinary sulfur, with respect to the inorganic, ethereal and neutral forms, was the same for heated and raw soybeans. However, in comparing the actual amounts of the different forms of sulfur excreted on the two diets, it is seen that a decrease in the excretion of total, inorganic, ethereal and neutral sulfur occurred on changing to the heated diet.

SUMMARY

Sulfur and nitrogen balances on rats confirmed previous findings that the nutritive value of soybean protein is improved by heat. The claim that treatment with certain solvents such as hexane and methanol have a similar effect was not substantiated. It appears that soybeans contain a S and N containing complex which is absorbable but cannot be used for tissue building purposes. Heating the soybeans makes it available.

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